Francine Bertolais do Valle Souza

Eficácia e Segurança do Rituximab no tratamento da Síndrome de Sjögren primária (SSp): Revisão sistemática e metanálise

Tese apresentada à Universidade Federal de São Paulo - Escola Paulista de Medicina, para obtenção do Título de Mestre em Ciências.

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Rituximab Effectiveness and Safety for Treating Primary Sjögren's Syndrome (pSS): Systematic Review and Meta-Analysis.

1. **Revisão Sistemática. 2. Metanálise. 3. Rituximab. 4. Síndrome de Sjögren primária.**

UNIVERSIDADE FEDERAL DE SÃO PAULO

ESCOLA PAULISTA DE MEDICINA

DEPARTAMENTO DE MEDICINA

DISCIPLINA DE MEDICINA DE URGÊNCIA E MEDICINA BASEADA EM EVIDÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE BASEADA EM EVIDÊNCIAS

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Francine Bertolais do Valle Souza

Eficácia e Segurança do Rituximab no Tratamento da Síndrome de Sjögren Primária (SSp): Revisão Sistemática e Metanálise.

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Dedicatória

À Deus, por me dar saúde e sabedoria para que eu pudesse sempre seguir seus caminhos e pelas maravilhosas oportunidades que me proporcionou até aqui.

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"Aqueles que se apaixonam pela prática sem a ciência são como um marinheiro que entra em um navio sem leme ou bússola e que nunca pode ter certeza do lugar para onde está indo.” (Leonardo da Vinci)

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Lista de Abreviaturas e Símbolos

DM Diferença de média.

ECR Ensaios Clínicos Randomizados.

ESSDAI *EULAR Sjögren's Syndrome Disease Activity Index.*

ESSPRI *EULAR Sjögren's Syndrome Patient Reported Index.*

EVA Escala Visual Analógica.

FACIT-F *Chronic Illness Therapy-Fatigue.*

FR Fator Reumatóide.

GRADE *Grading of Recommendations, Assessment, Development and Evaluations.*

IC Intervalo de Confiança.

PROFAD *Profile of Fatigue and Discomfort*.

RevMan *Review Manager.*

RR Risco Relativo.

RTX Rituximab.

SF-36 *Short Form-36.*

SS Síndrome de Sjögren.

SSp Síndrome de Sjögren primária.

RESUMO

Introdução: A Síndrome de Sjögren (SSp) primária é uma doença autoimune, sistêmica, que acomete as glândulas exócrinas e menos frequentemente órgãos internos, apresenta intensa infiltração linfoplasmocitária principalmente do epitélio dos tecidos envolvidos, levando a destruição e perda da função secretora. As opções terapêuticas incluem principalmente medidas sintomáticas e de suporte, e drogas imunossupressoras tradicionais não têm mostrado eficácia em ensaios clínicos randomizados. O rituximab (RTX) é um anticorpo quimérico anti-CD20, que conduz à depleção de células B por diversos mecanismos. Há evidências de que esta droga possa ser eficaz para o tratamento da SSp. O objetivo desta revisão sistemática foi avaliar a eficácia e segurança RTX para tratamento de SSp.

Métodos e resultados: Foi realizada uma revisão sistemática de ensaios clínicos randomizados publicados até dezembro de 2015, sem restrição de linguagem. Foram realizadas estratégias de busca nas seguintes bases de dados científicas: MEDLINE, EMBASE, CENTRAL e LILACS. Foram incluídos adultos com diagnóstico SSp estabelecido de acordo com os critérios de classificação Americano e Europeu 2002, e considerado o uso de RTX como a intervenção e o uso de outras drogas ou placebo como controle. Quatro estudos preencheram os critérios de elegibilidade: três com baixo risco de viés e um com risco incerto de viés. O número total de participantes foi de 276 (145 RTX, 131 placebo). Foi avaliado o risco de viés de cada estudo incluído e avaliados como desfechos principais: a função da glândula lacrimal, a função da glândula salivar, melhora a fadiga e eventos adversos. Não foram encontradas diferenças entre os grupos no teste de Schirmer na metanálise da semana 24 (DM 3,59, 95% IC -2,89 a 10,07). Apenas um estudo avaliou o teste de lissamina verde e relatou uma diferença estatisticamente significativa entre os grupos na semana 24 (DM -2,00, IC 95% -3,52 a -0,48). Houve diferença significativa entre os grupos em relação à taxa de fluxo salivar (DM 0,09, 95% IC 0,02-0,16) e melhora na fadiga através da EVA na semana 6 (RR 3,98, IC 95% 1,61-9,82) e na semana 16 (RR 3.08, 95 % IC 1,21-7,80).

Conclusões: De acordo com a evidência de qualidade moderada, o tratamento com um único ciclo (duas doses) de RTX em pacientes com SSp apresenta efeito discreto na função da glândula lacrimal. Evidências de baixa qualidade indicam o potencial desta droga para melhorar o fluxo salivar. De acordo com evidências de

baixa qualidade, não foram observadas diferenças na avaliação após 24 semanas relativas à redução de fadiga (30% da EVA), ocorrência de eventos adversos sérios, na qualidade de vida e atividade da doença. Com nível de evidência muito baixo, não houve melhora na secura da boca através da avaliação pela EVA.

1. INTRODUÇÃO

1.1 Revisão Sistemática

Revisão sistemática é um tipo de estudo planejado para avaliar as evidências e responder a uma pergunta específica. Utiliza métodos explícitos e sistemáticos para identificar, selecionar e avaliar criticamente os estudos, coletar e analisar os dados desses estudos incluídos na revisão. Os métodos estatísticos (metanálise) podem ou não ser utilizados na análise e na síntese dos resultados dos estudos incluídos; assim, a revisão sistemática utiliza toda essa estruturação para evitar viés em cada uma de suas partes. Uma revisão sistemática tenta reunir toda evidência empírica com critérios de elegibilidade pré-especificados, a fim de responder a uma pergunta de pesquisa específica. As principais características de uma revisão sistemática são: ter uma questão a ser respondida com critéiros claramente definidos, objetivos com critérios pré-definidos de elegibilidade para estudos a depender da pergunta e desfechos claros e previamente validados. (Atallah, Castro 1998; Higgins 2011)

1.2 Síndrome de Sjögren primária (SSp)

A Síndrome de Sjögren primária (SSp) é uma doença autoimune sistêmica, que envolve as glândulas exócrinas e órgãos internos, levando à destruição e perda da função secretora devido à intensainfiltração linfoplamocitária das glândulas envolvidas. (Moutsopoulos, Youinou 1991; Tzioufas, Vlachoyiannopoulos 2012)

Essa síndrome foi primeiramente descrita por Johann Mikulicz em 1892 como "Síndrome de Mikulicz" após a descrição da infiltração celular e atrofia acinar, em glândulas salivares de um agricultor com 42 anos de idade, com aumento da glândula parótida bilateral. Em 1933, Henrik Sjögren, oftalmologista sueco, usou pela primeira vez o termo "ceratoconjuntivite seca" para descrever o sintoma de olho seco diferenciando-o de "xeroftalmia", que se trata do sintoma de olho seco, devido à deficiência de vitamina A. (Morgan, Castleman 1953, Yi et al., 2016)

Fatores genéticos, hormonais e externos contribuem para o desenvolvimento desta doença multifatorial (Moutsopoulos, Youinou1991; Tzioufas, Vlachoyiannopoulos 2012; Tzioufas et al., 2012), que tem uma distribuição a nível mundial e afeta principalmente as mulheres, na proporção de 9:1. O pico de incidência da SSp é entre 40 e 60 anos de idade, embora SSp possa ocorrer em qualquer idade (Plesivcnik et al., 2004; Alamanos et al., 2006) e estima-se que sua prevalência seja entre 0,5% a 3,3%, apresenta-se como a segunda doença autoimune sistêmica mais comum após a artrite reumatóide. (Bowman et al., 2004; Shiboski et al., 2012; Flament et al., 2016; Yi et al., 2016) Os primeiros sinais e sintomas de SSp geralmente são inespecíficos e o diagnóstico pode demorar entre 6 a 10 anos para ser estabelecido. (Kassan, Moutsopoulos 2004)

Como outras doenças autoimunes, a compreensão dos mecanismos precisos que envolvem a etiopatogênese da SS ainda não foram totalmente esclarecidos, provavelmente fatores ambientais, vírus (Ebstein-Barr vírus, citomegalovírus, vírus linfotrópico de células T humanas tipo 1 - HTLV 1), parvovírus, vírus da hepatite C e vírus da imunodeficiência humana sejam as principais causas; além da predisposição genética (presença do alelo DQA1*0501) alterações hormonais (hipoestrogenismo e níveis baixos de de hidroepiandrosterona), levando a inflamação glandular inicial e uma resposta imune desregulada com ativação das respostas Th1 e Th2. (Flament et al., 2016)

Estudos têm procurado caracterizar a resposta imune que ocorre na síndrome, confirmando que as células B e T desempenham um papel importante nesta resposta imunitária, mas também realçam o papel importante da imunidade inata. O papel das células-T na SS tem sido extensivamente estudado e revisto com conclusões divergentes, especialmente em relação aos papéis das células T reguladoras e células T-*helper*, mas a ativação das células T citotóxicas é inegável. (Alunno et al., 2015; Chaigne et al., 2015)

A imunidade inata na SS é uma área de interesse crescente, destacando-se o papel importante do interferon (INF) permitindo-se dizer que a doença apresenta a “assinatura do interferon”. (Nezos et al., 2015) Novas descobertas sobre a imunidade inata também incluem defeito apoptótico na depuração celular, interação entre os receptores de P2X7 e inflamassoma e o papel das células

epiteliais, tal como outros elementos pró-inflamatórias, na resposta imune. (Baldini et al., 2015; Fragoulis et al., 2015; Luciano et al., 2015)

Evidências clínicas e experimentais suportam a importância dos linfócitos B na patogênese da SSp. O fator ativador do linfócito B (BAAF) apresenta níveis elevados no sangue, saliva e glândulas salivares desses pacientes. (Mariette et al., 2003; Pers et al., 2005) Níveis de BAAF relacionam-se com altos níveis de hipergamaglobulinemia e dos autoanticorpos anti-Ro/SSA e anti-La/SSB séricos e também na formação de centros germinativos glandulares. (Mariette et al., 2003; Gottenberg et al., 2005)

A ativação de células B inicialmente policlonal pode progredir para linfoproliferação monoclonal com o desenvolvimento do linfoma. Estudos demonstram risco relativo entre 16 a 44 de desenvolvimento de linfoma em pacientes com SSp. (Kassan et al., 1978; Nocturne et al., 2014)

Critérios de classificação podem ser usados para garantir a padronização do diagnóstico em pacientes que participam em estudos clínicos, e para facilitar a análise dos resultados ea comparação de pacientes entre instituições. (Vitali et al., 2016)

Entre 1988 e 1996, o Grupo Europeu de Estudo sobre critérios de classificação para SS realizou um estudo multicêntrico cujos objetivos foram: validar um questionário simples para sintomas de secura; selecionar os testes mais sensíveis e específicos para o diagnóstico de SS; definir um conjunto de critérios de classificação para esta doença; e, validar estes critérios definidos. Os objetivos do estudo foram alcançados, com a colaboração de diferentes centros europeus durante suas diversas fases e proporcionando um grande número de pacientes e controles. (Vitali et al., 2016)

Os critérios de classificação Americano/Europeu de 2002 para a classificação da SS (**Quadro1.**), têm sido utilizado em um grande número de estudos clínicos e citado em livros texto de medicina interna e reumatologia. No entando, nos últimos anos têm sofrido críticas e novos critérios foram idealizados como os 2012, apesar disso, os critérios de classificação de 2002 serão mantidos até que haja um consenso para a sua substituição. (Shiboski et al., 2012)

Quadro1. Critérios classificatórios internacionais revisados para SS/2002

1 - Sintomas oculares: uma resposta positiva para pelo menos uma das questões ✓ Você já teve olhos desconfortavelmente secos, diariamente, por mais de três meses? ✓ Você tem sensação recorrente de areia ou cascalho nos olhos? ✓ Você usa lágrimas artificiais mais do que três vezes ao dia?
2 - Sintomas orais: uma resposta positiva para pelo menos uma das questões ✓ Você já teve sensação diária de boca seca por mais de três meses? ✓ Você costuma ter aumento persistente ou recorrente das glândulas salivares na idade adulta? ✓ Você costuma beber líquidos para ajudar na deglutição de comidas secas?
3 - Sinais oculares – evidência objetiva do envolvimento ocular definido por pelo menos um dos testes positivo • Teste de Schirmer sem anestesia (≤ 5 mm em 5 minutos) • Escore de Rosa Bengala ou outro corante (≥ 4 de acordo com o sistema van Bijsterveld)
4 – Histopatologia • Biópsia de glândulas salivares menores mostrando sialoadenite linfocítica focal, com escore focal ≥ 1 Escore focal: número de focos linfocitários contendo mais de 50 linfócitos por 4 mm^2 de tecido glandular com mucosa acinar adjacente de aspecto normal
5 - Envolvimento de glândula salivar: evidência objetiva do envolvimento de glândula salivar pela positividade de pelo menos um dos seguintes testes • Fluxo salivar total não estimulado (≤ 1,5 ml em 15 min) • Sialografia de parótida com sialectasias (padrões puntato, cavitário ou destruído), sem evidência de obstrução dos ductos maiores • Cintilografia salivar mostrando retardo na captação, baixa concentração e/ou atraso na excreção do radiofármaco
6 – Autoanticorpos Presença do anticorpo anti-Ro/SSA e/ou anti-La/SSB
Regras para classificação SSp: presença de 4 dos 6 itens, contanto que o item 4 ou 6 seja positivo ou 3 dos 4 critérios objetivos. SSs: diagnóstico bem definido de outra doença do tecido conjuntivo, na presença dos itens 1 ou 2 associados a quaisquer 2 itens entre 3 e 5. Critérios de exclusão: história prévia de irradiação em cabeça/pescoço, infecção por hepatite C ou HIV, linfoma pré-existente, sarcoidose, doença do enxerto versus hospedeiro, uso de drogas anticolinérgicas.

Adaptado de Vitali et al., 2002

Essa síndrome caracteriza-se por secura das mucosas, principalmente ocular e oral. (Shiboskiet al., 2012) A doença pode se estender além do envolvimento das glândulas exócrinas e manifestações sistêmicas, incluindo fadiga, artralgia/artrite, vasculite, alterações pulmonares, doença renal, comprometimento neurológico, hepático e pancreático podem ocorrer, além desses pacientes terem um risco aumentado para desenvolvimento de linfoma.

(Fox 2005; Quartuccio et al., 2014, Flament et al., 2016) Pacientes com SSp apresentam amplo espectro de alterações nos exames laboratoriais, como citopenias, hipergamaglobulinemia, presença de anti-Ro/SSA, anti-La/SSB, fator antinuclear, fator reumatóide (FR), crioglobulinas e hipocomplementemia. (Retamozo, 2012)

O tratamento da SS é multidisciplinar e envolve a participação de educadores, nutricionistas, fisioterapeutas, terapeutas ocupacionais e médicos (em geral reumatologistas, oftalmologistas, otorrinolaringologistas, neurologistas, ginecologistas e hematologistas). Os objetivos do tratamento são: aliviar os sintomas da síndrome seca e melhorar a qualidade de vida (prevenir ou retardar a progressão da doença). (Thanou-Stavraki, James 2008; Tzioufas 2012) As opções terapêuticas incluem principalmente medidas sintomáticas e de suporte. (Ramos-Casals et al., 2010)

Imunossupressores e imunobiológicos (anti-fator de necrose tumoral) não demontraram eficácia em ensaios clínicos randomizados realizados em pacientes com SSp. (Skopouli et al., 1996; Mariette et al., 2004; van Woerkom et al., 2007)

O rituximab (RTX) é um anticorpo anti-CD20 quimérico, que conduz à depleção de células B por diversos mecanismos. Há evidências de que esta droga possa ser eficaz para o tratamento de SSp. (Thanou-Stavraki, James 2008) No entanto, os resultados de estudos sobre a eficácia do RTX são controversos, principalmente devido às diferentes manifestações clínicas. (Devauchelle-Pensec et al., 2007; Thanou-Stavraki, James 2008; Ramos-Casals et al., 2010; Meiners et al., 2011) Com objetivo de diminuir as incertezas com relação a efetividade do RTX no tratamento da SSp resolvemos realizar uma revisão sistemática da literatura.

2. MÉTODOS

2.1 Desenho de estudo

Foi realizada uma revisão sistemática da literatura de acordo com a metodologia da *Cochrane Collaboration* disponível no *Cochrane Handbook of Systematic Reviews of Interventions*. (Higgins, Green 2011)

Inicialmente, elaborados um protocolo de pesquisa aprovado, sem restrições, pelo Comitê de Ética em Pesquisa (CEP) da Universidade Federal de São Paulo/Escola Paulista de Medicina e registrado na Plataforma Brasil do Ministério da Saúde (**Apêndice I**).

2.2 Local

Estudo realizado no Programa de Pós-graduação em Saúde Baseada em Evidências (PGSBE), Escola Paulista de Medicina – Universidade Federal de São Paulo (UNIFESP).

2.3 Amostra

2.3.1 Tamanho da amostra

Foram incluídos todos os estudos encontrados com a estratégia de busca especificada e que preencheram os critérios de inclusão especificados a seguir.

2.3.2 Critérios de inclusão

2.3.2.1 Tipos de estudos

Apenas ensaios clínicos randomizados que preencheram os critérios de inclusão, independentemente do idioma e tipo de publicação.

Estudos “quase-randomizado”, coorte, caso-controle e transversal foram excluídos.

2.3.2.2 Tipos de participantes

Participantes com idade superior a 18 anos e com diagnóstico SSp estabelecido de acordo com os Critérios de Classificação Americano-Europeu, 2002. (Vitali et al., 2002)

2.3.2.3 Tipos de intervenção

Considerou-se o uso de RTX como a intervenção e o uso de outras drogas ou placebo como controle.

2.3.2.4 Tipos de desfechos

Para ser incluído, um estudo precisava apresentar pelo menos um dos seguintes desfechos:

2.3.2.4.1 Desfechos primários

1. Função da glândula lacrimal, avaliados através do teste de Schirmer, teste de lissamina verde, teste de fluoresceína, rosa bengala e escala visual analógica para secura ocular (EVA secura ocular).
2. Função da glândula salivar, avaliada através do fluxo salivar e escala visual analógica para secura oral (EVA secura oral).
3. Fadiga avaliada por meio da *Functional Assessment of Chronic Illness Therapy-Fatigue* (FACIT-F), *Profile of Fatigue and Discomfort* (PROFAD) e escala visual analógica (EVA fadiga).
4. Ocorrência de eventos adversos relatados pelos autores.

2.3.2.4.2 Desfechos secundários

1. Qualidade de vida, avaliada através do questionário de qualidade de vida Short Form-36 (SF-36) ou de outros instrumentos validados.
2. Atividade da doença, avaliada através do EULAR *Sjögren's Syndrome Disease Activity Index* (ESSDAI). (Serrano et al., 2013; Seror et al., 2015)
3. Alterações nas variáveis laboratoriais (linfócitos B, imunoglobulina e fator reumatóide).
4. Percepção dos sintomas, avaliada através do EULAR *Sjögren's Syndrome Patient Reported Index* (ESSPRI). (Seror et al., 2011)

2.4 Estratégia de busca dos estudos

Procuramos identificar todos os ECRs relevantes, independentemente do idioma e do *status* de publicação (publicados, não publicados, em impressão, ou em andamento).

Desenvolvemos estratégias de busca, incluindo os seguintes termos e sinônimos: *"rituximab", "CD20 antibody rituximab", "Mabthera", "Roche brand of rituximab", "rituxan", "Hoffmann-La Roche brand of rituximab", "IDEC brand of rituximab", "Genentech brand of rituximab", "IDEC-C2B8 antibody", "IDEC-C2B8", "Sjogren's Syndrome", "Sjogren Syndrome", "Sjogrens Syndrome", "Syndrome Sjogren's", "Sicca Syndrome"* e *"Syndrome Sicca".*

2.4.1 Busca eletrônica

Rodamos a estratégia de busca até dezembro de 2015 com filtros para RCTs nas seguintes bases eletrônicas: *Cochrane Central Register of Controlled Trials* (CENTRAL) (http://www.cochranelibrary.com/), MEDLINE (via PubMed)

(http://www.ncbi.nlm.nih.gov/pubmed/), EMBASE (http://store.elsevier.com/) e LILACS (http://lilacs.bvsalud.org/).

2.4.2 Busca manual

Realizamos busca manual nas listas de referências dos estudos relevantes e busca em literatura médica através da *Web of Science Proceedings*. A busca de ensaios em andamento e/ou não publicados foi realizada através das seguintes bases: *National Research Register*, *Early Warning System*, *Current Controlled Trials*, *MRC Clinical Trials database*, *The US National Institutes of Health Ongoing Trials Register* (www.clinicaltrials.gov).

2.5 Seleção dos estudos

Dois autores (FBVS e GJMP) rastrearam os estudos de forma independente. Através da leitura de títulos e resumos, avaliamos a elegibilidade dos estudos identificados.

Os estudos classificados como possivelmente elegíveis, foram lidos em texto completo e determinado à inclusão na revisão. Em caso de barreira da língua, submeteríamos os artigos a um tradutor qualificado e em caso de divergências de elegibilidade, um terceiro autor (VMFT) eliminaria a discordância. Os estudos classificados como não elegíveis tiveram suas razões especificadas.

2.5.1 Análise da qualidade metodológica dos estudos

Para esta avaliação foi utilizada a tabela de risco de viés da *Cochrane Collaboration* (**Quadro 2.**). (Higgins, Green 2011) De acordo com a tabela, a classificação do risco de viés é feita da seguinte maneira: “Sim” = baixo risco de viés; “Incerto” (não há informações suficientes para fazer o julgamento) = risco incerto de viés (risco moderado); “Não” = alto risco de viés; para cada um dos sete domínios: geração de sequência aleatória; ocultação da alocação; cegamento dosparticipantes e profissionais; cegamento dos avaliadores dos desfechos; desfechos incompletos; relato de desfecho seletivo e outras fontes de viés.

Cada ensaio clínico foi avaliado independentemente por dois revisores (FBVS e GJMP). A concordância interobservadores das relações e qualidade metodológica de cada estudo foi calculada através do teste estatístico de Kappa. (Cohen, 1960) Em caso de divergências, um terceiro autor (VMFT) eliminaria a discordância.

Quadro 2. Risco de viés (*Cochrane Collaboration*)

Critério	Descrição	Julgamento
Viés de seleção **1. Geração da sequência aleatória**	A sequência de alocação foi gerada de maneira adequada? Descreve o método utilizado para gerar a sequência de alocação com detalhes suficientes?	Sim Não Incerto
Viés de seleção **2. Ocultação da alocação**	A ocultação da alocação foi adequada? Descreve o método utilizado para ocultação da sequência de alocação com detalhes suficientes para determinar se a alocação da intervenção poderia ser conhecida antes ou durante o acompanhamento?	Sim Não Incerto
Viés de performace **3. Cegamento dos participantes e profissionais**	Descreve as medidas utilizadas para cegar os participantes do estudo quanto à intervenção recebida? O conhecimento da alocação da intervenção foi adequadamente prevenido durante o estudo? Informa se o mascaramentofoiefetivo?	Sim Não Incerto
Viés de detecção **4. Cegamento dos avaliadores dos desfechos**	Descreve todas as medidas utilizadas para cegar os avaliadores quanto à intervenção recebida pelos participantes? Informa se o mascaramento foi efetivo?	Sim Não Incerto
Viés de atrito **5. Desfechos incompletos**	Descreve todos os dados dos desfechos, incluindo perdas e exclusões na análise? Quando há perdas e exclusões, descreve o número em cada grupo de intervenção e razões? As perdas no seguimentoforam adequadamenterelatadas e analisadas?	Sim Não Incerto
Viés de relato **6. Relatode desfecho seletivo**	Os resultados do estudo são livres de sugestão de desfecho seletivo? Todos os desfechos propostos inicialmente (e/ou relevantes) foram relatados?	Sim Não Incerto

Outros viéses **7. Outrasfontes de viés**	O estudo está aparentemente livre de outros problemas que possam levar a algum risco de viés?	Sim Não Incerto

2.5.2 Extração de dados

Dois pesquisadores (FBVS e GJMP) extraíram os dados utilizando um formulário padronizado que continha informações sobre os participantes, intervenção, comparação, resultados e características do estudo **(Anexo 1)**.

2.6 Análise estatística

Foram incluídos os dados avaliados na 24ª semana, partindo da linha de base. Os programas de análise estatística contidos no pacote *Review Manager* 5.3.5, fornecido pela *Cochrane Collaboration*, foram utilizados.

Dados contínuos foram comparados como diferença de média (DM) ou diferença de média padronizada (DMP), em caso de uso de ferramentas diferentes para análise do mesmo defecho. Dados dicotômicos foram expressos como risco relativo (RR) com respectivo intervalo de confiança (IC) de 95%. Quando clinicamente significante, os dados dicotômicos foram convertidos em número necessário para tratar (NNT), a partir da diferença de risco (DR), sendo calculado como o inverso da diferença de risco.

Adotamos o valor de $p \leq 0.05$ nos resultados dos desfechos clínicos. (Higgins, 2002) A heterogeneidade estatística foi avaliada por meio do teste de inconsitência (I^2), variando de 0-100% incluindo o intervalo de confiança de 95%. (Higgins, 2002). O I^2 demonstra o percentual de variação total através dos estudos causados por heterogeneidade e foi utilizado para julgar o grau de consistência da evidência obtida.

Assumiu-se como heterogeneidade estatística quandoo I^2 fosse superior a 50%, utilizando para esses casos, modelo estatístico de efeito randômico. Para os casos em que o valor de I^2 fosse menore que 50%, utilizamos o modelo estatístico

de efeito fixo. Cabe ressaltar que o teste de heterogeneidade só é calculado quando a metanálise contém dois ou mais estudos.

As possíveis fontes de heterogeneidade foram planejadas para serem avaliadas por sensibilidade e análises de subgrupos como descrito a seguir. A estratégia de teste do funil ("*funnel plot*") foi planejada, mas não foi realizada devido ao baixo número de estudos incluídos.

Quando possível, os dados dos estudos foram sumarizados em gráficos de metanálise (síntese quantitativa), caso contrário, os resultados de cada estudo foram apresentados individualmente e de modo narrativo (síntese qualitativa).

2.7 Análise de subgrupos

Planejamos executar análises de subgrupos para explorar as diferenças de tamanho de efeito de acordo com as seguintes características:

• Comprometimento de um órgão específico;

• Protocolo de intervenção (ciclo, dosagem ou comparação com outras drogas);

• Tempo da doença;

• Idade.

2.8 Análise de sensibilidade

Planejamos executar análises de sensibilidade com o propósito de explorar a influência dos seguintes fatores sobre o efeito estimado:

1. Repetir a análise excluindo estudos não publicados;
2. Repetir a análise considerando a qualidade metodológica do estudo, como especificado acima;
3. Repetir a análise excluindo todos os estudos muito longos ou muito grandes para estabelecer quanto que eles dominam o resultado;

4. Repetir a análise excluindo estudos que utilizassem os seguintes filtros: critérios de diagnóstico, idioma da publicação, fonte de financiamento (indústria versus outros) e país onde o estudo foi conduzido.

Na presente versão desta revisão não foi possível executar as análises de subgrupos e de sensibilidade.

3. RESULTADOS DOS ESTUDOS INCLUÍDOS

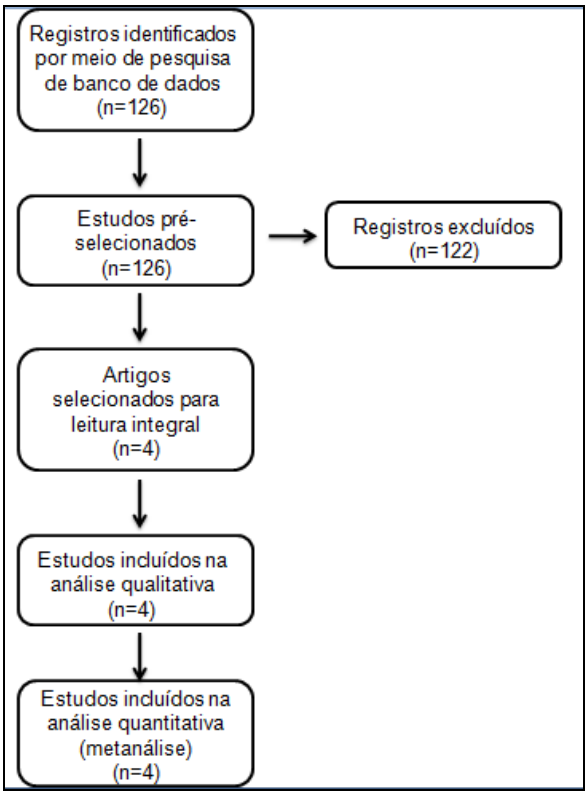
3.1 Descrição dos estudos

3.1.1 Estudos identificados

Foram identificados e selecionados 126 registros através da busca nas bases eletrônicas de dados. Após leitura de título e resumo, 122 foram excluídos por tratarem de assuntos não pertinentes ao estudo e quatro estudos (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014; Bowman et al., 2015) foram incluídos na análise qualitativa e quantitativa (metanálise) (**Figura 1**). Nenhum estudo foi identificado na busca manual e literatura cinzenta.

Os estudos incluídos totalizaram 276 participantes, sendo 145 no grupo RTX e 131 no grupo controle (placebo). Em todos os estudos incluídos (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014; Bowman et al., 2015) os pacientes foram randomizados para o grupo RTX (duas infusões 1g RTX: dia 1 e dia 15, curso único) ou grupo controle (infusão de placebo).

Figura 1. Fluxograma dos estudos selecionados.

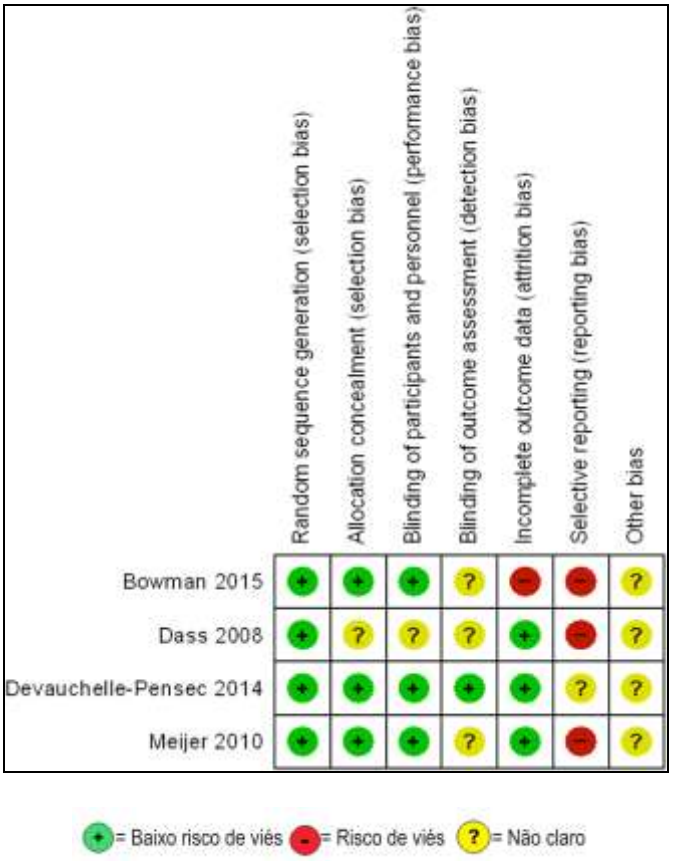


3.1.2 Qualidade metodológica

A análise de cada estudo incluído foi realizada por dois examinadores de maneira independente (GJMP e FBVS) através da ferramenta da *Cochrane Collaboration* para avaliar o risco de viés (**Figura 2**). Em caso de discordância um terceiro examinador seria solicitado (VFMT).

Entre os quatro estudos selecionados, três foram classificados como baixo risco de viés (Meijer et al., 2010; Devauchelle-Pensec et al., 2014; Bowman et al., 2015) e um estudo foi classificado como risco incerto de viés. (Dass et al., 2008)

Figura 2. Avaliação do risco de viés dos estudos incluídos.



3.1.3 Estudos excluídos

Foram excluídos 122 estudos após leitura de título e resumo. Os estudos foram excluídos por mais de uma razão, sendo a causa mais comum a ausência da associação entre a doença (SSp) e a intervenção (RTX) estabelecidos em nossa revisão.

3.1.4 Estudos incluídos

Quatro estudos atenderam aos nossos critérios de inclusão e foram incluídos em nossas análises. (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014; Bowman et al., 2015)

3.1.4.1 Desenho dos estudos incluídos

Os quatro estudos incluídos foram randomizados e controlados. A duração dos ensaios incluídos variou entre 24 (Dass et al., 2008; Devauchelle-Pensec et al., 2014) e 48 semanas. (Meijer et al., 2010; Bowman et al., 2015)

3.1.4.2 Tipos de participantes

Os quatro estudos incluídos totalizaram 276 participantes, sendo 145 no grupo RTX e 131 no grupo controle (placebo). A amostragem nos estudos variou de 17 (Dass et al., 2008) à 120 participantes (Devauchelle-Pensec et al., 2014), com idade entre 18 a 80 anos, com diagnóstico SSp estabelecido de acordo com os Critérios de Classificação Americano-Europeu, 2002. (Vitali et al., 2002)

3.1.4.3 Intervenções

Todos os estudos incluídos realizaram para o grupo RTX duas infusões de 1g RTX, no 1º e 15º dia ou infusão de placebo. Os dados foram analisados 24 semanas a partir da linha de base.

3.1.4.4 Dados faltantes

Utilizamos em nosso estudo técnicas de imputação de dados realizada estimando o desvio-padrão a partir do erro-padrão e do valor de P informado nos estudos obedecendo ao modelo proposto e recomendado pela *Cochrane Collaboration*, com ferramentas disponíveis no programa *Review Manager* (RevMan) (disponível em http://tech.cochrane.org/revman/download). (Higgins 2011)

Nos casos que mesmo assim, não foi possível obter os dados para a metanálise, os resultados foram apresentados de forma descritiva (síntese qualitativa) e discutidos no texto principal da revisão.

3.1.5 Minimização do viés de seleção

Todos os estudos descreveram adequadamente os procedimentos de randomização. (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2010; Bowman et al., 2015) O sigilo da alocação foi adequado em três estudos (Meijer et al., 2010; Devauchelle-Pensec et al., 2010; Bowman et al., 2015) e classificada como incerta para um dos estudos. (Dass et al., 2008)

3.1.6 Minimização do viés de perda

Perdas e exclusões foram descritas por todos. A abordagem de análise por intenção de tratar foi realizada quando necessária.

3.1.7 Minimização do viés de detecção

Apenas um estudo relatou que os avaliadores dos desfechos não estiveram cientes ao grupo para o qual os participantes foram alocados. (Devauchelle-Pensec et al., 2014) Três estudos não descreveram se os avaliadores dos desfechos estavam cientes quanto às alocações dos pacientes. (Dass et al., 2008; Meijer et al., 2010; Bowman et al., 2015)

3.1.8 Avaliação da força da evidência (GRADE)

Para a graduação da força das evidências obtidas a partir desta revisão, foi utilizada o GRADE (*Grading of Recommendations Assessment, Development and Evaluation*), que classifica as evidências como tendo qualidade alta, moderada, baixa ou muito baixa. (Guyatt et al., 2011) Os critérios para classificação levam em consideração o desenho do estudo, o risco de viés, a inconsistência dos dados, a subjetividade ("*indirectness*", ou evidências indiretas), a imprecisão e o viés de publicação. (Guyattet al., 2011)

4.RESULTADOS DA REVISÃO E METANÁLISE

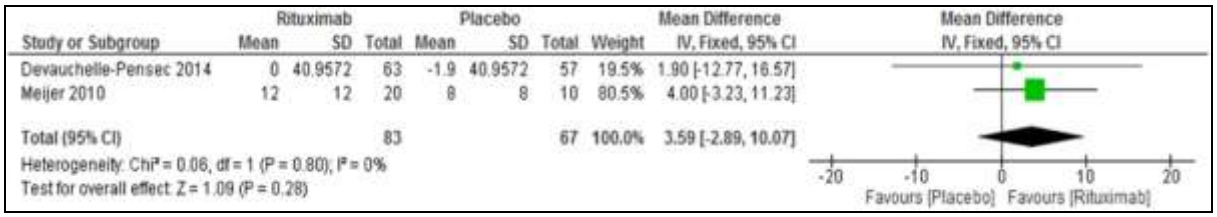
4.1 Desfechos primários

4.1.1 Função da glândula lacrimal

Três dos estudos incluídos avaliaram a função da glândula lacrimal através do teste de Schirmer. (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014) Dass et al.,(Dass et al., 2008) relataram que não houve nenhuma mudança, mas não apresentaram seus resultados. Foram incluídos apenas dois estudos (Meijer et al., 2010; Devauchelle-Pensec et al., 2014) na metanálise do desfecho teste de Schirmer em 24 semanas. Meijer et al., (Meijer et al., 2010) relataram melhora da função da glândula lacrimal no grupo RTX desde a linha de base até a semana 48, no entanto, não encontraram diferenças entre os grupos no teste de Schirmer. Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) não encontraram efeitos de RTX em variáveis associadas à secura, tais como os resultados para a produção lacrimal no teste de Schirmer. Realizamos um modelo metanálise de efeito fixo (I^2< 50%) para esse resultado e não observamos diferenças estatisticamente significativas entre o grupo RTX e grupo placebo na semana 24 (DM 3.59, IC 95%-2.89, 10.07; **Figura 3**).

Meijer et al., (Meijer et al., 2010) avaliaram a função da glândula lacrimal através do teste lisamina verde e encontraram uma diferença significativa entre o RTX e grupo placebo na semana 24 (DM -2.00, IC 95% -3.52, -0.48). Os autores também relataram melhora das pontuações da EVA para secura ocular no grupo RTX desde a linha de base até a semana 48, ao passo que a pontuação no grupo placebo só apresentou melhora significativa após a semana 5. Houve uma diferença significativa na alteração média de secura ocular avaliada através da EVA entre os grupos desde o início até a semana 24 (DM -27.00, IC 95% -46.28, -7.72), semana 36 (DM -24.0, IC 95% -44.5, -3.5) e semana 48(DM -30.00, IC 95% - 47.01, -12.99).

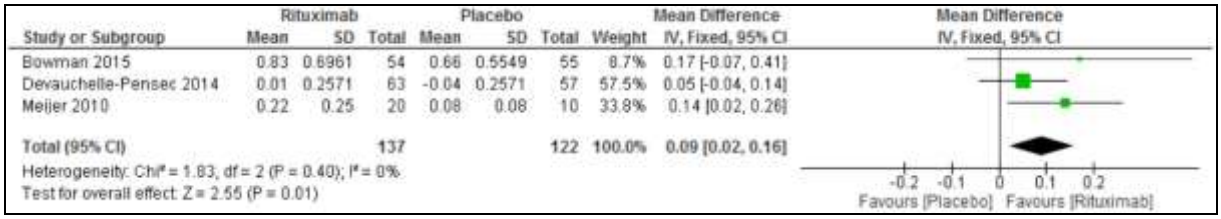
Figura 3. Rituximab X Placebo. Metanálise do desfecho teste de Schirmer na semana 24.



4.1.2 Função da glândula salivar

Todos os estudos incluídos realizaram análise de dados do fluxo salivar. (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014; Bowman et al., 2015) Dass et al.,(Dass et al., 2008) relataram que não houve nenhuma mudança, mas não apresentaram dados quantitativos, portanto, foram excluídos desta metanálise. (Dass et al., 2008) Meijer et al., (Meijer et al., 2010) relataram melhora de fluxo salivar no grupo RTX na semana 24 (DM 0.14, IC 95% 0.02, 0.26), enquanto Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) e Bowman et al., (Bowman et al., 2015) não observaram diferenças significativas entre o grupo RTX e placebo na semana 24 (DM 0.05, IC 95% -0.04, 0,14 e DM0.17, IC 95% -0.07, 0.41, respectivamente). Realizamos uma metanálise da taxa do fluxo salivar, com modelo de efeito fixo (I^2< 50%), que demonstrou uma diferença estatisticamente significativa entre os grupos a favor do grupo RTX na semana 24 (DM 0.09, IC95% 0.02, 0.16; **Figura 4**).

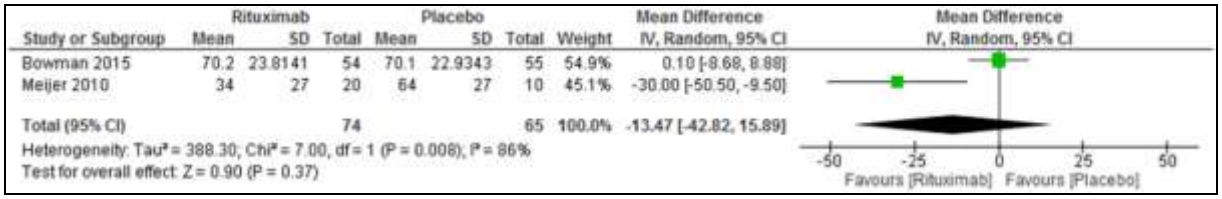
Figura 4. Rituximab X Placebo. Metanálise do desfecho taxa do fluxo salivar na semana 24.



Dois dos estudos incluídos (Meijer et al., 2010; Bowman et al., 2015), avaliaram secura da boca através da EVA. Meijer et al., (Meijer et al., 2010) relataram melhora na pontuação da EVA para todos os sintomas de secura oral no grupo de RTX. Eles encontraram uma diferença média estatisticamente significativa entre os grupos na semana 24 (DM -30.00, IC 95% -50.50 a -9.50). Foi realizada uma metanálise de efeito randômico (I^2 > 50%) para esse desfecho e não foram observadas diferenças estatisticamente significativas entre os grupos na semana 24 (DM -13.47, IC 95% -42.82 a 15.89; **Figura 5**). Bowman et al., (Bowman et al., 2015) também avaliaram secura da boca através de taxas de porcentagem da EVA e não encontraram diferenças significativas entre os grupos (RR 0.93, IC 95% 0.45-1.93).

Segundo a abordagem GRADE, a metanálise do desfecho secura da boca avaliada através da EVA apresentou qualidade de evidência muito baixa.

Figura 5. Rituximab X Placebo. Metanálise do desfecho secura oral (EVA) na semana 24.



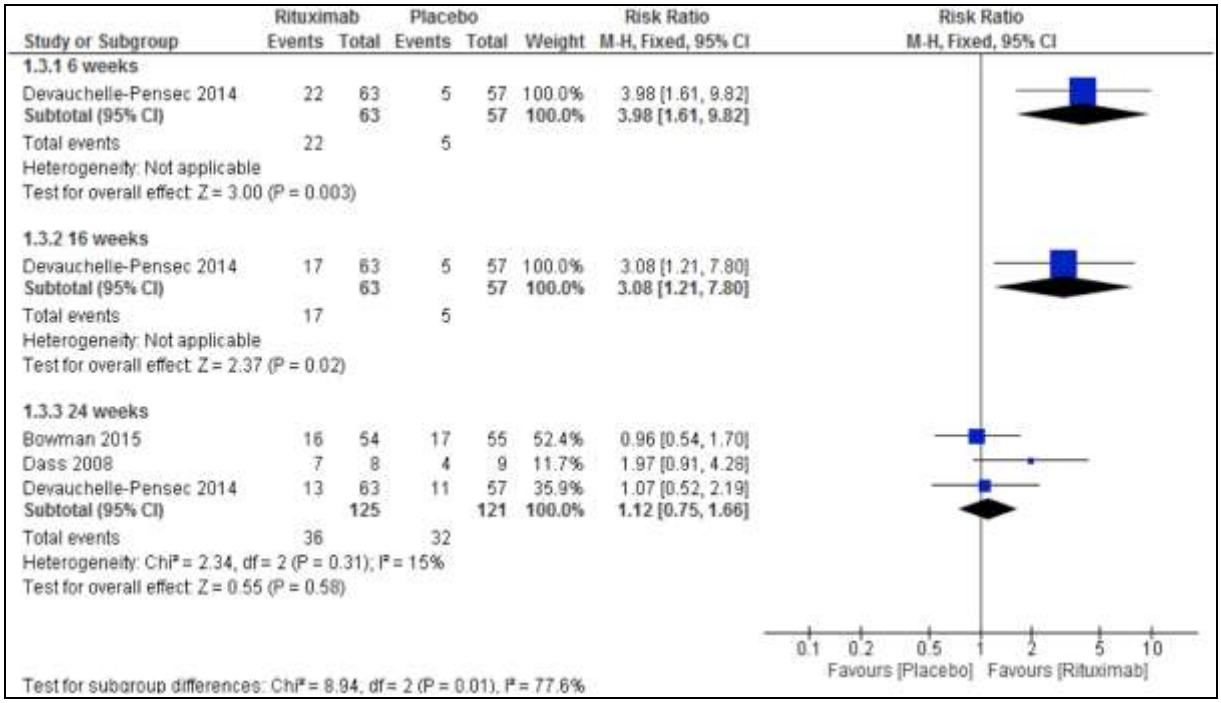
4.1.3 Fadiga

Três dos estudos incluídos avaliaram a fadiga através de EVA. Dass et al., (Dass et al., 2008), Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) e Bowman et al., (Bowman et al., 2015). Estabelecemos para esse estudo, uma melhora de até 30% da EVA como o objetivo primário. Não foram encontradas diferenças significativas entre os grupos na metanálise de melhora de até 30% da fadiga através da EVA na semana 24 (RR 1.12, IC 95% 0.75 a 1.66; **Figura 6**). No entanto, os resultados de Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) indicaram uma resposta favorável ao RTX na semana 6 (RR 3.98, IC95% 1.61 a 9.82; **Figura 6**) e na semana 16 (RR 3.08, IC 95% 1.21 a 7.80; **Figura 6**).

Bowman et al., (Bowman et al., 2015), também relataram resultados de fadiga através da pontuação média da EVA (0-100mm, sendo 100=grave) e não encontraram diferenças significativas entre os grupos (DM 5.0, IC 95% -3.37 a 13.37). Apenas Dass et al., (Dass et al., 2008) avaliaram o desfecho fadiga através do questionário SF-36 e os autores relataram que houve uma melhora significativano domínio fadiga no grupo RTX na semana 24 (p = 0,009), mas não no grupo placebo (p = 0,087).

Foi avaliada a qualidade da evidência através da abordagem GRADE. (Guyatt et al., 2011) A metanálise do teste de Schirmer apresentou qualidade moderada. As metanálises relecionadas à taxa de fluxo salivar e melhora da fadiga de até 30% através da EVA apresentaram evidências de baixa qualidade.

Figura 6. Rituximab X Placebo. Metanálise do desfecho melhor a de até 30% da fadiga avaliada através da EVA na semana 24.



4.1.4 Eventos adversos

Todos os estudos incluídos relataram efeitos adversos. Reações relacionadas à infusão, como calafrio, erupção cutânea macular e púrpura foram

mais frequentes no grupo RTX, assim como transtornos gastrintestinais, respiratórios e alterações musculoesqueléticas. No entanto, Meijer et al., (Meijer et al., 2010) e Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) encontraram taxas de infecção similares entre os grupos RTX e placebo.

Meijer et al. (Meijer et al., 2010) e Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) também relataram, respectivamente, que um e dois pacientes do grupo RTX desenvolveram púrpura dentro de 15 dias após a infusão de RTX. Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) relataram uma ocorrência no grupo placebo.

Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) também relataram eventos adversos como falta de ar, tosse seca, espirros ou irritação na garganta em sete pacientes RTX, e encontraram uma diferença significativa entre os grupos na proporção de doentes com pelo menos um distúrbio respiratório 24 horas após a infusão ($p = 0,014$). Apenas um desses eventos foi considerado grave e todos os pacientes melhoraram após diminuição da infusão, ou a interrupção do tratamento. Um paciente no grupo do placebo apresentou um ataque de asma 15 dias após a infusão. (Devauchelle-Pensec et al., 2014)

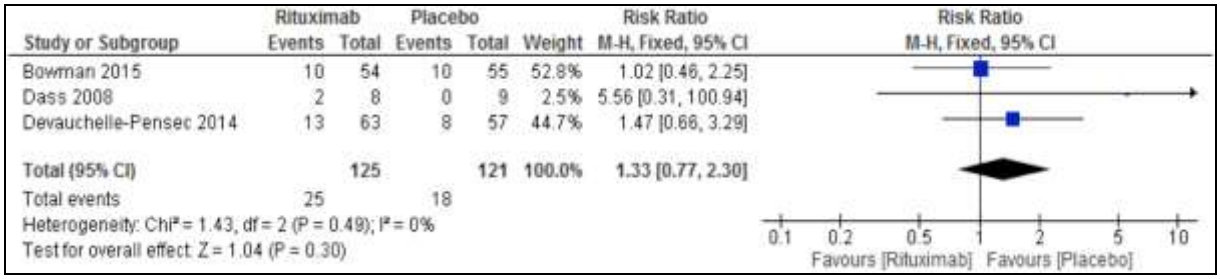
Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) relataram dois diagnósticos de câncer no grupo RTX durante as investigações: um no dia 7 a partir da linha de base (carcinoma de células escamosas da pele) e outro no dia 38 (câncer de mama, e a paciente foi à óbito um ano após a inclusão no estudo). Um paciente do grupo placebo foi diagnosticado com carcinoma basocelular superficial 125 dias após a inclusão no estudo. (Devauchelle-Pensec et al., 2014)

Bowman et al., (Bowman et al. 2015) relataram ocorrência de mais eventos adversos no grupo RTX do que no placebo (325 RTX e 275 placebo). No entanto, os eventos adversos considerados graves eram iguais entre os grupos (10 RTX e 10 placebo). Os autores também relataram apenas uma reação à infusão considerada grave (grupo RTX) e um anafilaxia grave (grupo placebo).

Eventos adversos graves ocorreram em 25 participantes do grupo RTX (Dass et al., 2008; Devauchelle-Pensec et al., 2014; Bowman et al. 2015) e em 18 participantes do grupo placebo. (Devauchelle-Pensec et al., 2014; Bowman et al. 2015) Foi realizada uma metanálise de efeito fixo ($I^2 < 50\%$) para este resultado e

não foram encontradas diferenças significativas entre os grupos (RR 1.33, IC 95% 0.77 a 2.30; **Figura7**). Esta metanálise apresentou evidências de baixa qualidade segundo a abordagem GRADE. (Guyattet al., 2011)

Figura7. Rituximab X Placebo. Metanálise do desfecho eventos adversos graves na semana 24.



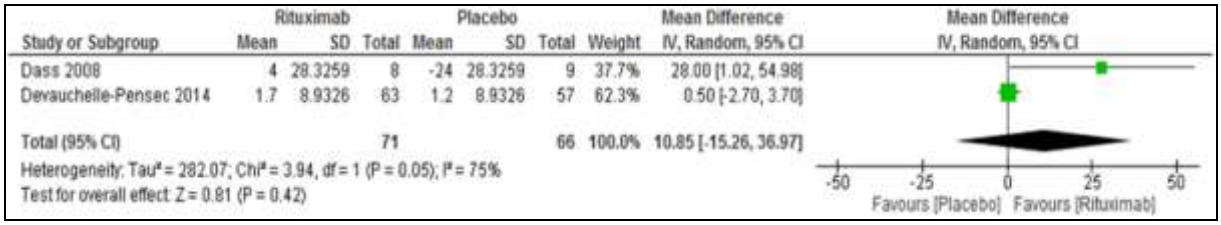
4.2 Desfechos secundários

4.2.1 Qualidade de vida

Três dos estudos incluídos (Dass et al., 2008; Meijer et al., 2010; Devauchelle-Pensec et al., 2014) avaliaram a qualidade de vida através do questionário de saúde SF-36, mas os resultados foram reportados de formas diferentes. Meijer et al., (Meijer et al., 2010) relataram apenas a pontuação total do SF-36 e segundo os autores, não encontraram diferenças significativas entre os grupos dede o início até a semana 48 (DM -5.00, IC 95% -17.15 a 7.15). Dass et al., (Dass et al., 2008) apenas relataram a ocorrência de melhora no domínio da saúde mental.

Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) descreveu o *score* do domínio saúde mental e domínio físico, mas não mencionou a pontuação total do SF-36. Os autores não encontraram diferenças significativas entre os grupos no domínio físico na semana 24 (DM 0.60, IC 95% -16.90 a 18.10). Devido à heterogeneidade entre os estudos, foi realizada uma metanálise de efeito randômico (I^2> 50%) dos resultados do domínio saúde mental na semana 24 e não foram encontradas diferenças significativas entre os grupos (DM 10.85, IC 95% -15.26 a 36.97; **Figura 8**). Esta metanálise apresentou evidências de baixa qualidade segundo a abordagem GRADE. (Guyattet al., 2011)

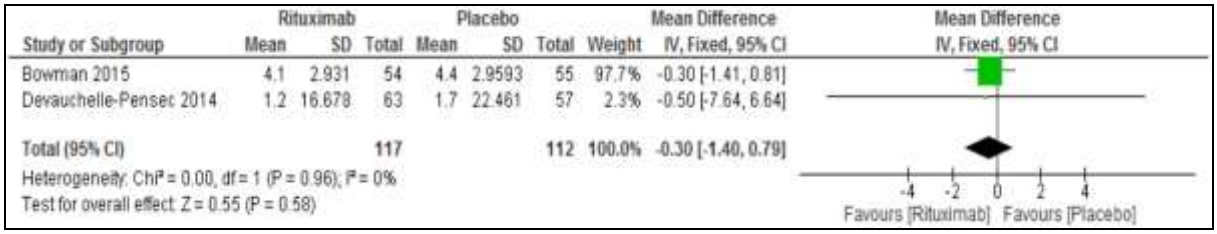
Figura 8. Rituximab X Placebo. Metanálise do questionário SF-36 domínio saúde mental na semana 24.



4.2.2 Atividade da doença

Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) e Bowman et al., (Bowman et al., 2015) avaliaram a atividade da doença através da ESSDAI. Foi realizada uma metanálise para este resultado e não foram encontradas diferenças significativas entre o RTX e grupo placebo (DM -0.30, IC 95% -1.40 a 0.79; **Figura 9**). De acordo com a abordagem GRADE, esta metanálise apresentou evidências de baixa qualidade. (Guyatt et al., 2011)

Figura 9. Rituximab X Placebo. Metanálise do desfecho atividade da doença avaliada através da ESSDAI na semana 24.



4.2.3 Alterações nas variáveis laboratoriais

Dass et al., (Dass et al., 2008) demonstraram que, em termos de resultados laboratoriais, houve uma diferença significativa na redução do FR na semana 24 favorável ao grupo RTX (DM 45, IC 95% 3.62 a 86.38). Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) mencionaram que iriam analisar

o desfecho FR como resultado secundário, no entanto, o resultado não foi apresentado no estudo.

Apenas Meijer et al., (Meijer et al., 2010) descreveram a análise do número de células B e observaram uma redução expressiva na média do número absoluto de células B após a primeira infusão de RTX sem alterações significativas no grupo do placebo. Os autores também encontraram diferenças estatisticamente significativas entre os grupos para esse desfecho desde linha de base até semanas 5, 12, 24, 36 e 48 (DM -0.23, IC 95% -0.31 a -0.15).

Dois estudos avaliaram os níveis de imunoglobulina. (Dass et al., 2008; Devauchelle-Pensec et al., 2014) Dass et al., (Dass et al., 2008) relataram uma diferença na redução percentual favorável ao RTX (p = 0,05). Devauchelle-Pensec et al., (Devauchelle-Pensec et al., 2014) encontraram uma redução significativa IgM no grupo RTX (DM 0.30, IC 95% 0.13 a 0.47).

4.2.4 Percepção dos sintomas

Apenas Bowman et al., (Bowman et al., 2015) avaliaram a percepção dos sintoma através do ESSPRI e não encontraram diferenças significativas entre o RTX e grupos placebo (DM 0.50, IC95% -0.19 a 1.19).

5. DISCUSSÃO

O número de artigos publicados sobre o anticorpo quimérico anti-CD20 (RTX) no tratamento da SSp vêm crescendo ao londo do tempo. No entanto, a maioria dos estudos identificados são relatos ou série de casos. Dos estudos encontrados, apenas quatro preencheram os critérios de inclusão para nosso estudo, sendo que um dos estudos (Bowman et al., 2015) foi publicado na forma de resultados preliminares. Carubbi et al. (Carubbi et al., 2013) publicaram um estudo comparando o RTX versus imunossupressores e encontraram resultados favoráveis ao RTX. No entanto, devido a aspectos metodológicos, este estudo não pôde ser incluído nesta revisão.

Até o momento, o tratamento da SSp incluiu apenas medidas sintomáticas e de suporte. ECRs com imunossupressores tradicionais não mostraram eficácia no tratamento desta doença multisistêmica. (Dross et al., 1986; Skopouli et al., 1996; Price et al., 1998; van Woerkom et al., 2007; Gottenberg et al., 2014) Devemos também levar em consideração que essa resposta insatisfatória em parte, pode ser devido ao tempo para a realização do diagnóstico, tornando muitas lesões irreversíveis e não responsivas aos tratamentos propostos até então.

O conhecimento sobre a frequência de características sistêmicas graves, a probabilidade de evolução para doenças linfoproliferativas (Zintzaras et al., 2005), o progresso no reconhecimento do papel das citocinas (tais como IFN-γ e IL-2) concomitante com a ativação anormal da célula B demonstra ser uma possibilidade de novas medidas terapêuticas. (Groom et al., 2002; Mariette et al., 2003; Szodoray et al., 2004)

Pacientes com SSp apresentam hiperatividade das células B, o que sugere um papel importante destas células na patogênese da síndrome. (Groom et al., 2002; Mariette et al., 2003) Sabe-se que o RTX atua diretamente contra estas células, e é considerado um tratamento potencial para SSp.

Ainda não há um consenso sobre o melhor intervalo de tempo para avaliar a eficácia do tratamento SSp. (Devauchelle-Pensec et al., 2014) Consideramos um período de tratamento de 24 semanas para análise dos resultados, uma vez que este intervalo foi comum a todos os estudos incluídos.

As manifestações sistêmicas não foram avaliadas nos estudos e o período de acompanhamento foi curto, não sendo possível analisar fatores de prognóstico da doença. Nossos principais resultados foram sobre a função da glândula ocular, salivar e fadiga. Dois estudos (Devauchelle-Pensec et al., 2014; Bowman et al., 2015) avaliaram a atividade da doença e não encontraram diferenças significativas entre os grupos. A ação do RTX na redução dos níveis séricos de FR e de células B foi demonstrada por Dass et al. (Dass et al., 2008) e Meijer et al. (Meijer et al., 2010), respectivamente.

Os pacientes com SSp possuem alta incidência de linfoma não-Hodgkin e linfomas, principalmente na mucosa. (Ioannidis et al., 2002; Voulgarelis et al., 2012) O uso de RTX, associada ou não à quimioterapia, parece ser uma opção para o tratamento nesses casos de complicações da SSp. (Voulgarelis et al., 2012) Entrentanto, uma vez que os estudos incluídos não relataram a presença de pacientes com linfoma, não foi possível concluir sobre este aspecto.

Um aumento significativo do risco de efeitos adversos, principalmente reações à infusão e distúrbios respiratórios foram observados para os participantes submetidos ao tratamento com RTX em comparação com os que receberam placebo em todos os estudos incluídos. No entanto, as taxas de infecção foram semelhantes entre os grupos em três estudos (Dass et al., 2008; Meijer et al., 2010; Devauchele-Pensec et al.,2014) e a ocorrência de eventos adversos considerados graves também. (Bowman et al., 2015)

Esta avaliação destaca a dificuldade e inadequação da investigação na SSp uma vez que existem apenas alguns estudos randomizados comparando a eficácia destra droga em relação ao placebo ou outras drogas. Os estudos incluídos apresentaram desfechos semelhantes, no entanto, a avaliação dos resultados ainda necessita de padronização, por esse motivo, em nosso estudo, não foi possível realizar uma análise de sensibilidade ou subgrupos.

Dentre as limitações do estudo podemos citar a avaliação de apenas um ciclo de tratamento com RTX, o número de participantes incluídos, mesmo nas metanálises, e ausência da análise para eficácia em diferentes manifestações sistêmicas. Em relação aos benefícios observados, somente os resultados para o desfecho fluxo salivar demonstraram evidência de melhora, e RTX demonstrou

ser uma droga segura uma vez que não houve diferenças na presença de eventos adversos graves, comparados ao grupo placebo. No entanto para efeito de segurança o tempo de 24 semanas pode não ser o mais adequado, períodos de seguimento mais longos são necessários. Para a prática clínica, é necessário ponderar os benefícios e malefícios desta intervenção no tratamento da SSp.

6. CONCLUSÕES

De acordo com evidências de qualidade moderada, o tratamento com um único curso de RTX em pacientes com SSp apresenta efeito discreto para melhorar a função da glândula lacrimal. Evidências de baixa qualidade indicam o potencial desta droga para melhorar o fluxo salivar e de acordo com evidências de baixa qualidade, não foram observadas diferenças na avaliação após 24 semanas relativas à redução de fadiga (30% da EVA), ocorrência de eventos adversos graves, melhora da qualidade de vida e atividade da doença. Com nível de evidência muito baixo nível, não houve melhora na avaliação da secura oral através da EVA.

7. ANEXOS

Anexo 1. Formulário de extração de dados.

Data collection form

Intervention review – RCTs

This form can be used as a guide for developing your own data extraction form. Sections can be expanded and added, and irrelevant sections can be removed. It is difficult to design a single form that meets the needs of all reviews, so it is important to consider carefully the information you need to collect, and design your form accordingly. Information included on this form should be comprehensive, and may be used in the text of your review, 'Characteristics of included studies' table, risk of bias assessment, and statistical analysis.

Notes on using a data extraction form:

- Be consistent in the order and style you use to describe the information for each report.
- Record any missing information as unclear or not described, to make it clear that the information was not found in the study report(s), not that you forgot to extract it.
- Include any instructions and decision rules on the data collection form, or in an accompanying document. It is important to practice using the form and give training to any other authors using the form.

Review title or ID	
Study ID *(surname of first author and year first full report of study was published e.g. Smith 2001)*	
Report ID	
Report ID of other reports of this study	
Notes	

General Information

Date form completed *(dd/mm/yyyy)*	
Name/ID of person extracting data	
Reference citation	
Study author contact details	
Publication type *(e.g. full report, abstract, letter)*	
Notes:	

Study eligibility

Study Characteristics	Eligibility criteria:	Eligibility criteria met? Yes	No	Unclear	Location in text or source *(pg & ¶/fig/table/other)*
Type of study	Randomised Controlled Trial	☐	☐	☐	
	Cluster or Cross-over study	☐	☐	☐	
Participants	**Diagnostic criteria for the participants** Participants older than 18 years and established pSS diagnosis according to the Classification Criteria American-European, 2002. (Vitali et al., 2002)	☐	☐	☐	
Types of intervention	**RTX versus another drugs or placebo**	☐	☐	☐	
Types of outcome measures		☐	☐	☐	
INCLUDE ☐		EXCLUDE ☐			
Reason for exclusion					
Notes:					

DO NOT PROCEED IF STUDY EXCLUDED FROM REVIEW

Characteristics of included studies

Methods

	Descriptions as stated in report/paper	**Location in text or source** *(pg & ¶/fig/table/other)*
Aim of study *(e.g. efficacy, equivalence, pragmatic)*		
Design*(e.g. parallel, crossover, non-RCT)*		

Unit of allocation *(by individuals, cluster/ groups or body parts)*			
Start date			
End date			
Duration of participation *(from recruitment to last follow-up)*			
Ethical approval needed/ obtained for study	☐ Yes ☐ No ☐ Unclear		
Notes:			

Participants

	Description: *Participants older than 18 years and established pSS diagnosis according to the Classification Criteria American-European, 2002. (Vitali et al., 2002)*		Location in text or source *(pg & ¶/fig/table/other)*
Population description *(from which study participants are drawn)*			
Setting *(including location and social context)*			
Inclusion criteria			
Exclusion criteria			
Method of recruitment of participants *(e.g. phone, mail, clinic patients)*			
Informed consent obtained	☐ Yes ☐ No ☐ Unclear		
Total no. randomised *(or total pop. at start of study for NRCTs)*			

Clusters *(if applicable, no., type, no. people per cluster)*		
Baseline imbalances		
Withdrawals and exclusions *(if not provided below by outcome)*		
Age		
Sex		
Race/Ethnicity		
Severity of illness		
Co-morbidities		
Other relevant sociodemographics		
Subgroups measured		
Subgroups reported		
Notes:		

Outcomes
Copy and paste table for each outcome.
Outcome 1

lacrimal gland function *(evaluated through the Schirmer test, lissamine green or fluorescin test and VAS)*	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table/other)*
Outcome name		
Time points measured *(specify whether from start or end of intervention)*		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/ reporting		
Unit of measurement *(if relevant)*		

Scales: upper and lower limits *(indicate whether high or low score is good)*		
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear	
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Power *(e.g. power & sample size calculation, level of power achieved)*		
Notes:		

Outcome 2

salivary gland function *(evaluated through salivary flow rate and VAS)*	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table/other)*
Outcome name		
Time points measured *(specify whether from start or end of intervention)*		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/ reporting		
Unit of measurement *(if relevant)*		
Scales: upper and lower limits *(indicate whether high or low score is good)*		

Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		
Imputation of missing data *(e.g. assumptions made for ITT analysis)*			
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*			
Power *(e.g. power & sample size calculation, level of power achieved)*			
Notes:			

Outcome 3

Fatigue *(evaluated through the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), VAS and the Profile of Fatigue and Discomfort (PROFAD)).*	Description as stated in report/paper		Location in text or source *(pg & ¶/fig/table/other)*
Outcome name			
Time points measured *(specify whether from start or end of intervention)*			
Time points reported			
Outcome definition *(with diagnostic criteria if relevant)*			
Person measuring/ reporting			
Unit of measurement *(if relevant)*			
Scales: upper and lower limits *(indicate whether high or low score is good)*			
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear		

Imputation of missing data *(e.g. assumptions made for ITT analysis)*		
Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Power *(e.g. power & sample size calculation, level of power achieved)*		
Notes:		

Outcome 4

adverse events reported by authors	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table/other)*
Outcome name		
Time points measured *(specify whether from start or end of intervention)*		
Time points reported		
Outcome definition *(with diagnostic criteria if relevant)*		
Person measuring/ reporting		
Unit of measurement *(if relevant)*		
Scales: upper and lower limits *(indicate whether high or low score is good)*		
Is outcome/tool validated?	☐ Yes ☐ No ☐ Unclear	
Imputation of missing data *(e.g. assumptions made for ITT analysis)*		

Assumed risk estimate *(e.g. baseline or population risk noted in Background)*		
Power *(e.g. power & sample size calculation, level of power achieved)*		
Notes:		

Risk of Bias assessment

See Chapter 8 of the Cochrane Handbook. Additional domains may be added for non-randomised studies.

Domain	Risk of bias Low High Unclear	Support for judgement *(include direct quotes where available with explanatory comments)*	Location in text or source *(pg & ¶/fig/table/other)*
Random sequence generation *(selection bias)*	☐ ☐ ☐		
Allocation concealment *(selection bias)*	☐ ☐ ☐		
Blinding of participants and personnel *(performance bias)*	☐ ☐ ☐	Outcome group: All/	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	Outcome group:	
Blinding of outcome assessment *(detection bias)*	☐ ☐ ☐	Outcome group: All/	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	Outcome group:	
Incomplete outcome data *(attrition bias)*	☐ ☐ ☐	Outcome group: All/	
(if separate judgement by outcome(s) required)	☐ ☐ ☐	Outcome group:	

Selective outcome reporting? *(reporting bias)*	☐ ☐ ☐		
Other bias	☐ ☐ ☐		
Notes:			

Data and analysis

Copy and paste the appropriate table for each outcome, including additional tables for each time point and subgroup as required.

Dichotomous outcome

	Description as stated in report/paper				Location in text or source *(pg & ¶/fig/table/other)*
Comparison					
Outcome					
Subgroup					
Time point *(specify from start or end of intervention)*					
Results	Intervention		Comparison		
	No. with event	Total in group	No. with event	Total in group	
Any other results reported *(e.g. odds ratio, risk difference, CI or P value)*					
No. missing participants					
Reasons missing					
No. participants moved from other group					
Reasons moved					
Unit of analysis *(by individuals, cluster/groups or body parts)*					

Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*			
Reanalysis required? *(specify, e.g. correlation adjustment)*	☐ Yes ☐ No ☐ Unclear		
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear		
Reanalysed results			
Notes:			

For RCT/CCT
Continuous outcome

	Description as stated in report/paper					Location in text or source *(pg & ¶/fig/table/other)*
Comparison						
Outcome						
Subgroup						
Time point *(specify from start or end of intervention)*						
Post-intervention or change from baseline?						
Results	Intervention			Comparison		
	Mean	SD *(or other variance, specify)*	No. participants	Mean	SD *(or other variance, specify)*	No. Participants
Any other results reported *(e.g. mean difference, CI, P value)*						
No. missing participants						
Reasons missing						

No. participants moved from other group			
Reasons moved			
Unit of analysis *(individuals, cluster/ groups or body parts)*			
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*			
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear		
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear		
Reanalysed results			
Notes:			

Continuous outcome

	Description as stated in report/paper						Location in text or source *(pg & ¶/fig/table /other)*
Comparison							
Outcome							
Subgroup							
Time point *(specify from start or end of intervention)*							
Post-intervention or change from baseline?							
Results	Intervention			Comparison			
	Mean	SD *(or other variance, specify)*	No. participants	Mean	SD *(or other variance, specify)*	No. participants	

Any other results reported *(e.g. mean difference, CI, P value)*			
No. missing participants			
Reasons missing			
No. participants moved from other group			
Reasons moved			
Unit of analysis *(individuals, cluster/ groups or body parts)*			
Statistical methods used and appropriateness of these *(e.g. adjustment for correlation)*			
Reanalysis required? *(specify)*	☐ Yes ☐ No ☐ Unclear		
Reanalysis possible?	☐ Yes ☐ No ☐ Unclear		
Reanalysed results			
Notes:			

Other outcome

	Description as stated in report/paper	Location in text or source *(pg & ¶/fig/table /other)*
Comparison		
Outcome		
Subgroup		

<table>
<tr><td>Time point
(specify from start or end of intervention)</td><td colspan="4"></td><td></td></tr>
<tr><td rowspan="2">No. participants</td><td colspan="2">Intervention</td><td colspan="2">Control</td><td rowspan="2"></td></tr>
<tr><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td rowspan="4">Results</td><td>Intervention result</td><td>SE (or other variance)</td><td>Control result</td><td>SE (or other variance)</td><td rowspan="4"></td></tr>
<tr><td></td><td></td><td></td><td></td></tr>
<tr><td colspan="2">Overall results</td><td colspan="2">SE (or other variance)</td></tr>
<tr><td colspan="2"></td><td colspan="2"></td></tr>
<tr><td>Any other results reported</td><td colspan="4"></td><td></td></tr>
<tr><td>No. missing participants</td><td colspan="2"></td><td colspan="2"></td><td></td></tr>
<tr><td>Reasons missing</td><td colspan="2"></td><td colspan="2"></td><td></td></tr>
<tr><td>No. participants moved from other group</td><td colspan="2"></td><td colspan="2"></td><td></td></tr>
<tr><td>Reasons moved</td><td colspan="2"></td><td colspan="2"></td><td></td></tr>
<tr><td>Unit of analysis (by individuals, cluster/groups or body parts)</td><td colspan="4"></td><td></td></tr>
<tr><td>Statistical methods used and appropriateness of these</td><td colspan="4"></td><td></td></tr>
<tr><td>Reanalysis required? (specify)</td><td>☐ Yes ☐ No ☐ Unclear</td><td colspan="3"></td><td></td></tr>
<tr><td>Reanalysis possible?</td><td>☐ Yes ☐ No ☐ Unclear</td><td colspan="3"></td><td></td></tr>
<tr><td>Reanalysed results</td><td colspan="4"></td><td></td></tr>
<tr><td colspan="6">Notes:</td></tr>
</table>

Other information

	Description as stated in report/paper	**Location in text or source** *(pg & ¶/fig/table/other)*
Key conclusions of study authors		
References to other relevant studies		
Correspondence required for further study information*(from whom, what and when)*		
Notes:		

Definitions

Assumed risk estimate	An estimate of the risk of an event or average score without the intervention, used in Cochrane 'Summary of findings tables'. If a study provides useful estimates of the risk or average score of different subgroups of the population, or an estimate based on a representative observational study, you may wish to collect this information.
Bias	A systematic error or deviation in results or inferences from the truth. In studies of the effects of health care, the main types of bias arise from systematic differences in the groups that are compared (selection bias), the care that is provided, exposure to other factors apart from the intervention of interest (performance bias), withdrawals or exclusions of people entered into a study (attrition bias) or how outcomes are assessed (detection bias). Reviews of studies may also be particularly affected by reporting bias, where a biased subset of all the relevant data is available.
Change from baseline	A measure for a continuous outcome calculated as the difference between the baseline score and the post-intervention score.
Clusters	A group of participants who have been allocated to the same intervention arm together, as in a cluster-randomised trial, e.g. a whole family, town, school or patients in a clinic may be allocated to the same intervention rather than separately allocating each individual to different arms.
Co-morbidities	The presence of one or more diseases or conditions other than those of primary interest. In a study looking at treatment for one disease or condition, some of the individuals may have other diseases or conditions that could affect their outcomes.

Compliance	Participant behaviour that abides by the recommendations of a doctor, other health care provider or study investigator (also called adherence or concordance).
Contemporaneous data collection	When data are collected at the same point(s) in time or covering the same time period for each intervention arm in a study (that is, historical data are not used as a comparison).
Exclusions	Participants who were excluded from the study or the analysis by the investigators.
Imputation	Assuming a value for a measure where the true value is not available (e.g. assuming last observation carried forward for missing participants).
Integrity of delivery	The degree to which the specified procedures or components of an intervention are delivered as originally planned.
Post-intervention	The value of an outcome measured at some time point following the beginning of the intervention (may be during or after the intervention period).
Power	In clinical trials, power is the probability that a trial will obtain a statistically significant result when the true intervention effect is a specified size. For a given size of effect, studies with more participants have greater power. Note that power should not be considered in the risk of bias assessment.
Providers	The person or people responsible for delivering an intervention and related care, who may or may not require specific qualifications (e.g. doctors, physiotherapists) or training.
Quasi-randomised controlled trial	A study in which the method of allocating people to intervention arms was not random, but was intended to produce similar groups when used to allocate participants. Quasi-random methods include: allocation by the person's date of birth, by the day of the week or month of the year, by a person's medical record number, or just allocating every alternate person.
Reanalysis	Additional analysis of a study's results by a review author (e.g. to introduce adjustment for correlation that was not done by the study authors).
Report ID	A unique ID code given to a publication or other report of a study by the review author (e.g. first author's name and year of publication). If a study has more than one report (e.g. multiple publications or additional unpublished data) a separate Report ID can be allocated to each to help review authors keep track of the source of extracted data.
Sociodemographics	Social and demographic information about a study or its participants, including economic and cultural information, location, age, gender, ethnicity, etc.

Study ID	A unique ID code given to an included or excluded study by the review author (e.g. first author's name and year of publication from the main report of the study). Although a study may have multiple reports or references, it should have one single Study ID to help review authors keep track of all the different sources of information for a study.
Theoretical basis	The use of a particular theory (such as theories of human behaviour change) to design the components and implementation of an intervention
Unit of allocation	The unit allocated to an intervention arm. In most studies individual participants will be allocated, but in others it may be individual body parts (e.g. different teeth or joints may be allocated separately) or clusters of multiple people.
Unit of analysis	The unit used to calculate N in an analysis, and for which the result is reported. This may be the number of individual people, or the number of body parts or clusters of people in the study.
Unit of measurement	The unit in which an outcome is measured, e.g. height may be measured in cm or inches; depression may be measured using points on a particular scale.
Validation	A process to test and establish that a particular measurement tool or scale is a good measure of that outcome.
Withdrawals	Participants who voluntarily withdrew from participation in a study before the completion of outcome measurement.

Sources:

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Anexo 2. Artigos incluídos na revisão.

Extended report

Reduction of fatigue in Sjögren syndrome with rituximab: results of a randomised, double-blind, placebo-controlled pilot study

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ABSTRACT

Objective: Primary Sjögren syndrome (pSS) causes significant systemic symptoms including fatigue as well as glandular dysfunction. There are currently no effective systemic therapies; however, open label series have suggested that rituximab may be beneficial for systemic and glandular manifestations. Therefore, we performed a double blind, placebo-controlled, randomised pilot study of the efficacy of rituximab in reducing fatigue in pSS.

Methods: A total of 17 patients with pSS and a score on fatigue visual analogue scale (VAS) >50 were randomised to receive either 2 infusions of rituximab 1 g or placebo; patients also received oral and intravenous steroids. Outcome measures included: the proportion of patients with >20% reduction in fatigue VAS, changes in pSS related symptoms, health related quality of life and immunological parameters of pSS. These were measured 6 months after therapy.

Results: There was significant improvement from baseline in fatigue VAS in the rituximab group ($p<0.001$) in contrast to the placebo group ($p = 0.147$). There was a significant difference between the groups at 6 months in the social functioning score of SF-36 ($p = 0.01$) and a trend to significant difference in the mental health domain score of SF-36 ($p = 0.06$). There was one episode of serum sickness in the rituximab treated group.

Conclusions: This is the first double blind study of rituximab in pSS to show benefit; further studies are justified.

Primary Sjögren syndrome (pSS) is a chronic autoimmune disorder affecting 0.2–3% of the population.[1] Although the hallmark of the disorder is chronic inflammation of the salivary and lacrimal glands, systemic manifestations are common. Fatigue is prominent among these, has been found to be a major cause of disability for patients,[2] and it is also regarded as a key symptom by doctors.[3] The 36-item Short Form health questionnaire (SF-36) has identified substantially reduced health related quality of life in these patients and validated specific and sensitive tools have been designed to study fatigue in pSS.[4]

There is currently no proven effective systemic therapy for pSS. Neither corticosteroids nor disease modifying antirheumatic drugs (DMARDs) have been shown to have a significant effect on the disease course.[5 6] Randomised, controlled studies have also failed to show significant differences between placebo and the anti-tumour necrosis factor (TNF) agents, infliximab and etanercept.[7 8]

The presence of autoantibodies, however, including anti-Ro, anti-La and rheumatoid factor (RF) as well as hyper γ-globulinaemia indicate a degree of B cell hyperactivity. Furthermore, B cell infiltrates, particularly of memory B cells, have been identified in salivary gland biopsy specimens.[9]

Recent open label studies have indicated efficacy of B cell depletion with rituximab, a chimaeric anti-CD20 monoclonal antibody first developed for the treatment of B cell lymphoma, a noted complication of pSS.[10] Use of a variety of doses of rituximab, outcome measures (glandular and extraglandular) and timepoints[11 12] led to the conclusion that randomised controlled trials were warranted in pSS. Furthermore, the efficacy of rituximab in rheumatoid arthritis[13] and connective tissue diseases[14] often associated with SS, suggests further clinical as well as theoretical grounds for use in pSS.

We therefore undertook an exploratory, pilot, randomised, double-blind, placebo controlled trial in order to determine the effect size of rituximab and thus whether a larger study would be feasible; this was in addition to assessment of the efficacy and safety of rituximab in pSS.

PATIENTS AND METHODS

This was a double-blind, randomised pilot study of rituximab vs placebo in 17 patients. The protocol was approved by the local ethics committees and all patients gave written, informed consent. Patients were enrolled at two sites in the UK.

Patients were eligible for the study if they fulfilled the American–European Consensus Criteria for pSS.[1] Patients were required to be positive for either anti-Ro and/or anti-La antibody and to have scored >50 on a 100 mm fatigue visual analogue scale (VAS) (ranging from 0 for absence of fatigue to 100 for worst imaginable fatigue). Patients were excluded if they had active, concurrent depression or anxiety, other autoimmune disease or a history of solid organ malignancy.

Baseline evaluations included medical history, physical examination, routine haematology, biochemistry, hepatitis serology, electrocardiogram and chest radiograph. The following investigations were carried out to assess baseline disease activity: fatigue assessed by VAS and the Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) questionnaire;[15] quality of life by SF-36 questionnaire;[16] specific Sjögren related symptoms (for somatic fatigue, mental fatigue and sicca symptoms) by Profile of Fatigue and Discomfort

(PROFAD) questionnaire[9] (this is made up of domains scored individually; these scores can be added in various combinations eg, combined fatigue score (aggregate of somatic and mental fatigue), sicca score (aggregate of oral, ocular, skin and dryness) or combined fatigue and discomfort score (PROFAD = fatigue+arthralgia+vascular dysfunction)); Schirmer-1 test; unstimulated salivary flow rate test; erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). These investigations were repeated at monthly intervals after therapy for 6 months. RF and serum immunoglobulins were measured at baseline and 6 months.

Patients randomised to active therapy received two infusions of 1 g rituximab on days 1 and 15. Each infusion was preceded by 100 mg methylprednisolone, intravenously. Randomisation was performed by use of a computer-generated list, stratified by site. Following reports of serum sickness in patients with pSS treated with rituximab[11] and after 1 serious adverse event in the second patient in this study (described below), 12 of the remaining patients received oral prednisolone between days 2–14, at a dose of 60 mg daily for days 2–7 and 30 mg on days 8–14. Patients could continue with concurrent medication but were prohibited from changing or adding disease modifying therapy during the course of the study. Patients were reviewed on a monthly basis for 6 months after therapy with a subsequent visit at 12 months after therapy for safety purposes.

This was a pilot study carried out to enable larger, controlled studies to be appropriately powered. The primary efficacy outcome was a 20% improvement in fatigue VAS score, 6 months after therapy. Secondary efficacy outcomes were derived from the measures of activity described above. Statistical analysis was carried out by t test for baseline data; χ^2 test for primary outcome; t test for mean improvement in fatigue VAS and for laboratory outcomes (ESR, CRP, RF, serum Igs). Non-parametric statistical tests (Mann–Whitney U) were carried out for questionnaire derived outcome measures. As this was a pilot study, no formal power calculation in designing this trial was felt to be appropriate.

RESULTS

A total of 18 patients were recruited and full 6-month data are available for 17 subjects; 8 were randomised to receive rituximab and 9 received placebo. Baseline characteristics did not differ significantly between the two groups (table 1). Of note were high scores on fatigue VAS, 76 vs 69 mm and relatively long disease duration, 7.25 vs 8.25 years (for rituximab vs placebo). None of the patients had other significant systemic complications of pSS.

At 6 months, seven of eight patients receiving rituximab (87.5%) and five of nine patients receiving placebo (55.6%) demonstrated >20% improvement in fatigue VAS (χ^2, p = 0.36) (fig 1). The mean improvement in fatigue VAS at 6 months was 49.5% (rituximab) vs 20.5% (placebo) (t test, p = 0.24). Using 30% improvement from baseline as a threshold for defining response, the number of rituximab responders was unchanged but fewer placebo patients (four of nine) achieved response (p = 0.064).

There was significant improvement from baseline in fatigue VAS in the rituximab group (mean (SD) improvement 36.8, (17.9), p<0.001) in contrast to the placebo group (mean improvement 17.3, (32.2), p = 0.147) (fig 2). General health VAS also improved significantly in the rituximab group (p = 0.021) but not in the placebo group (p = 0.96). Change in fatigue over time between the two groups indicated that the rituximab treated patients had greater reduction in fatigue than the placebo group at each month between treatment and 6 months afterwards (fig 3). The somatic fatigue domain of the PROFAD showed significant improvement in the rituximab treated group (p = 0.009) but not in the placebo group (p = 0.087). There was also a significant difference between baseline and 6 months in the rituximab treated group in PROFAD outcome (p = 0.026) but not in the placebo group (p = 0.219).

There was a significant difference at 6 months in the social functioning score of SF-36 (mean improvement in score, 12 vs −25, rituximab vs placebo, p = 0.01) and a trend to significant difference in the mental health domain score of SF-36 (mean improvement in score, 4 vs −24, rituximab vs placebo, p = 0.06). Patients treated with rituximab showed improvement in the mental component summary of the SF-36[12] whereas patients who received placebo had deterioration (−8) (p = 0.06). No significant difference was observed in change in the physical health component of the SF-36 or in pain VAS.

The change in fatigue VAS was much more variable in the placebo group (fig 4). In the placebo group, the change varied between approximately 99% improvement and 110% worsening (interquartile range 78%). In the active group the change varied between 83% improvement and 11% worsening (interquartile range 59%). In terms of laboratory outcomes, there was a significant difference in the reduction of RF between the two groups at 6 months (45 vs 0, rituximab vs placebo, p = 0.05) but no significant change was observed in immunoglobulin levels or titres or positivity for other antibodies. As only one patient in

Table 1 Baseline characteristics

	Rituximab	Placebo
n	8	9
Age at baseline (range)	51 (22–64)	54 (41–64)
Median disease duration, years (range)	7.25 (1–18)	8.25 (2–19)
Median fatigue VAS, mm (range)	76 (61–95)	69 (53–89)
IgG, g/litre (range)	18.61 (12.03–29.35)	20.98 (7.11–38.54)
Anti-Ro	100%	100%
Anti-La	78%	67%
SF-36 PCS (range)	43.6 (24–56)	36.7 (13–80)
SF-36 MCS (range)	42.6 (16–62)	52.1 (25–83)

PCS, physical component score; MCS, mental component score; SF-36, 36-item Short Form health survey; VAS, visual analogue scale.

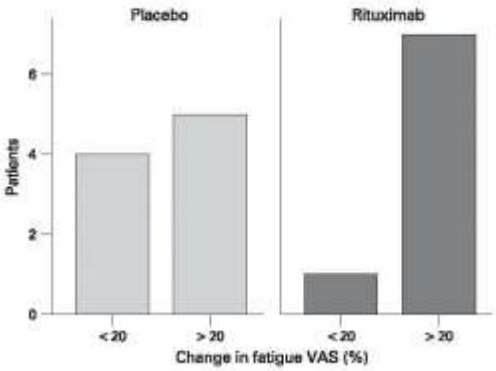

Figure 1 Number of patients meeting primary endpoint (≥20% reduction in fatigue visual analogue scale (VAS)).

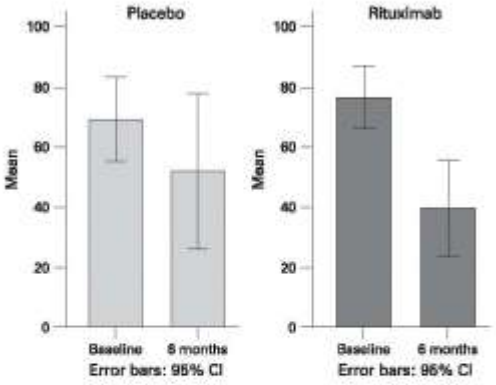

Figure 2 Mean fatigue visual analogue scale at baseline and 6 months.

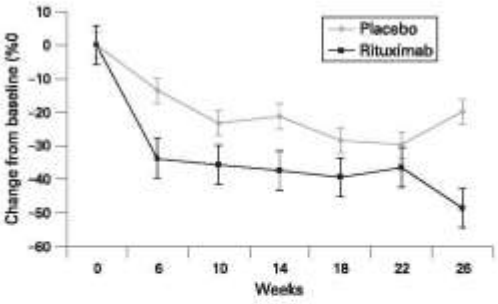

Figure 3 Change in fatigue visual analogue scale from baseline.

the active treatment arm did not respond, no specific analysis of whether baseline characteristics at baseline influenced outcome was undertaken. However, this non-responder did have less reduction in RF after treatment (16.7%) than the responders (mean reduction in RF 42.0% (21.8), range 17.4–67.9%)). This patient also had less reduction in immunoglobulin levels with 10.99% reduction in IgM, 2% reduction in IgG and 0.8% rise in IgA. Of the responders following rituximab, mean (SD) reduction in all immunoglobulins was noted: IgM 30.82% (21.99); IgG 12.42% (8.51); IgA 10.09% (13.11).

No significant differences in glandular manifestations of pSS were observed at 6 months. There was no significant change in the Schirmer-1 test score or in unstimulated salivary flow rate at that timepoint. Following rituximab, seven of eight patients had B cell depletion $<0.005\times10^9$/litre. At 6 months, B cell numbers were detectable above this level in seven of eight patients. There was no correlation between presence of B cells at 6 months and clinical status (at that timepoint).

Safety

Three serious adverse events (SAEs) occurred in two patients in the rituximab group. One patient developed symptoms of headache, urticarial rash, fever and meningism 7 days after the first infusion of rituximab. This was the second patient treated in the study and so she did not receive oral steroids as per the initial protocol. Infective meningitis was excluded and a diagnosis of serum sickness was made. The patient responded well to intravenous steroids. As a result of this episode, the study protocol was amended to include 2 weeks of oral steroid therapy (details above) between the two infusions of rituximab.

One other patient suffered two SAEs; the first was an admission to hospital for 24 h with abdominal pain, eventually diagnosed as gastroenteritis (4 weeks after the second infusion of rituximab) and this patient was also admitted to hospital for observation for palpitations for 24 h, 3 months after therapy. No significant cause for these symptoms was found.

Two patients in the rituximab group also experienced infusion reactions; these were both during the first infusion and consisted of rigors and a macular rash. The infusions were restarted and completed uneventfully after administration of antihistamine and hydrocortisone.

Pilot statistical analysis

Analysis of this pilot data indicates that a study with 37 patients in each arm would have adequate power to investigate the outcome used in this study ie, the hypothesis that rituximab therapy would lead to a 20% improvement in fatigue VAS score, 6 months after therapy over and above the improvement following placebo therapy.

DISCUSSION

This is the first randomised, double-blind, placebo controlled study of rituximab in treating fatigue in Sjögren syndrome. These results suggest that rituximab can improve fatigue in pSS. Rituximab also improved aspects of quality of life in pSS, with significant improvement in the social functioning domain score of SF-36 and a trend to significant improvement in the mental health domain score of SF-36. Rituximab therapy also significantly reduced RF levels over placebo without reducing overall Ig levels. This is the first therapy to show benefit over placebo in this disease and also indicates that fatigue is an important outcome measure that can be investigated usefully.

This study investigated improvement in fatigue as a primary outcome. Other studies of biological agents in pSS have used sicca symptoms or composite outcomes (sometimes including fatigue) as a primary outcome measure. The rationale for studying fatigue is that with pain it contributes more to quality of life than dryness.[17] However, fatigue often has composite causes, including psychosocial. In this study, we attempted to minimise confounding factors for fatigue by excluding patients with active depression and anxiety and we screened for other disorders known to contribute to fatigue, such as thyroid disease.

This trial was designed as a pilot study. Patients receiving rituximab demonstrated greater mean improvement in fatigue although the results show a relatively high placebo effect for the 20% improvement threshold. It is calculated that a study with 37 patients in each arm would be adequately powered for this endpoint; by comparison, a pilot study of etanercept vs placebo in pSS[9] calculated that an adequately powered study would require 288 subjects in total (albeit for different outcome, namely a composite of sicca symptoms and inflammatory marker/immunoglobulins). The "placebo" effect may be related to the role of steroids that were introduced following the occurrence of a serum sickness type reaction and reports of similar reactions in other studies. The dose of steroid used was identical to that in concurrent studies of rituximab in rheumatoid arthritis. In this study, patients treated with rituximab/steroids had greater reduction in fatigue than

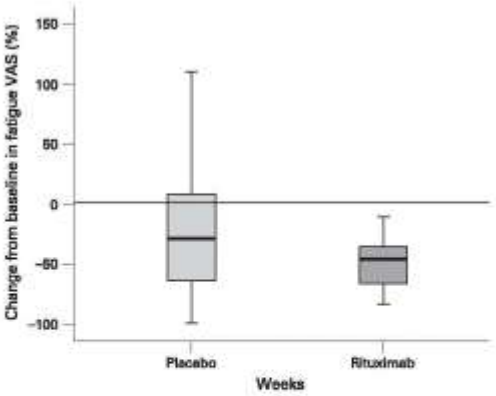

Figure 4 Change in fatigue visual analogue scale (VAS) from baseline to 6 months, demonstrating greater variability in placebo group.

placebo/steroids only treated patients 1 month after therapy and this remained the case at each month thereafter. At 6 months, the effect of placebo/steroids was markedly variable, with striking improvement and deterioration. The improvement in the rituximab group was generally of a greater magnitude than in the placebo group and there was less variability supporting our hypothesis that rituximab has a beneficial effect on fatigue in pSS in patients who are positive for anti-Ro or anti-La autoantibodies. This greater improvement in the rituximab treated group was present immediately after treatment; at the week 6 timepoint, rituximab treated patients had 33% improvement in fatigue vs 15% in patients treated with steroid and placebo. This suggests that rituximab has a beneficial effect over and above that of oral prednisolone and the placebo group results at 6 months may simply be due to a true placebo effect. Limited longer-term data (at 12 months post therapy) is available (eight patients, four on rituximab, four placebo). These data indicate that responses to rituximab are sustained in some patients (58.7% improvement in fatigue VAS at 6 months vs 58.4% at 12 months) in contrast, patients receiving placebo had less improvement in fatigue VAS (35.5%) at 12 months.

Open label studies have suggested benefit in glandular manifestations of pSS but with increased disease duration, glandular atrophy rather than dysfunction is the major cause of dryness. In an open label series, those with lowest disease duration were more likely to show improvement in sicca symptoms after rituximab. Our patients had relatively long disease duration (7 vs 8 years, rituximab vs placebo) and thus change in sicca symptoms was perhaps less likely.

The safety profile of rituximab was generally satisfactory. No striking differences in the rates of infection or other adverse effects were seen between the two groups. Episodes of serum sickness have been reported previously only after the use of rituximab in Sjögren syndrome. The increased incidence of serum sickness in this group of patients is not entirely understood but it may be that the development and subsequent deposition of immune complexes is more likely because of hyper γ-globulinaemia in this population. The occurrence of infusion reactions and serum sickness may suggest that administration of rituximab with concomitant steroids should still be standard practice. Although rheumatoid factor fell, no reduction in overall immunoglobulin levels was seen, which is reassuring for safety, at least in the short term. This study also only enrolled patients who were positive for anti-Ro and/or anti-La antibodies; as this is in the light of data that rituximab was more effective in patients with RA positive for rheumatoid factor and/or anti-CCP antibodies, further studies of rituximab in pSS ought to focus on patients who are autoantibody positive.

The data from this pilot study suggest, therefore, that rituximab may well offer benefit for patients with Sjögren syndrome related fatigue and indicate that an adequately powered randomised study is feasible and justified.

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Competing interests: None declared.

Ethics approval: The protocol was approved by the local ethics committees and all patients gave written, informed consent.

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Reduction of fatigue in Sjögren syndrome with rituximab: results of a randomised, double-blind, placebo-controlled pilot study

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Effectiveness of Rituximab Treatment in Primary Sjögren's Syndrome

A Randomized, Double-Blind, Placebo-Controlled Trial

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Objective. **To study the efficacy and safety of B cell depletion with rituximab, a chimeric murine/human anti-CD20 monoclonal antibody, in patients with primary Sjögren's syndrome (SS) in a double-blind, randomized, placebo-controlled trial.**

Methods. **Patients with active primary SS, as determined by the revised American–European Consensus Group criteria, and a rate of stimulated whole saliva secretion of ≥0.15 ml/minute were treated with either rituximab (1,000 mg) or placebo infusions on days 1 and 15. Patients were assigned randomly to a treatment group in a ratio of 2:1 (rituximab:placebo). Followup was conducted at 5, 12, 24, 36, and 48 weeks. The primary end point was the stimulated whole saliva flow rate, while secondary end points included functional, laboratory, and subjective variables.**

Results. **Thirty patients with primary SS (29 female) were randomly allocated to a treatment group. The mean ± SD age of the patients receiving rituximab was 43 ± 11 years and the disease duration was 63 ± 50 months, while patients in the placebo group were age 43 ± 17 years and had a disease duration of 67 ± 63 months. In the rituximab group, significant improvements, in terms of the mean change from baseline compared with that in the placebo group, were found for the primary end point of the stimulated whole saliva flow rate ($P = 0.038$ versus placebo) and also for various laboratory parameters (B cell and rheumatoid factor [RF] levels), subjective parameters (Multidimensional Fatigue Inventory [MFI] scores and visual analog scale [VAS] scores for sicca symptoms), and extraglandular manifestations. Moreover, in comparison with baseline values, rituximab treatment significantly improved the stimulated whole saliva flow rate ($P = 0.004$) and several other variables (e.g., B cell and RF levels, unstimulated whole saliva flow rate, lacrimal gland function on the lissamine green test, MFI scores, Short Form 36 health survey scores, and VAS scores for sicca symptoms). One patient in the rituximab group developed mild serum sickness–like disease.**

Conclusion. **These results indicate that rituximab is an effective and safe treatment strategy for patients with primary SS.**

Sjögren's syndrome (SS) is a systemic autoimmune disease that is characterized by chronic inflammation of the salivary and lacrimal glands, resulting in xerostomia and keratoconjunctivitis sicca in ~95% of patients (1). These symptoms are frequently accompanied by extraglandular manifestations such as Raynaud's phenomenon, arthritis, arthralgia, and myalgia, and 85% of patients experience severe fatigue. Moreover, B cell hyperactivity, reflected by increased serum levels of IgG and IgM rheumatoid factor (RF) and the presence of anti-SSA and anti-SSB autoantibodies, is a common finding in SS. Furthermore, SS has a large impact on health-related quality of life, employment, and disability, as reflected by lower Short Form 36 (SF-36) health survey scores, reduced employment rates, and higher rates of disability in patients with SS compared with the general population (1).

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To date, no targeted systemic treatment has been available for SS. In pilot trials, however, it has been shown that rituximab, a chimeric murine/human anti-CD20 monoclonal antibody that binds to the B cell surface antigen CD20, might improve subjective and objective symptoms related to primary SS for at least 6–9 months (2,3). On the basis of these promising results, a randomized, double-blind, placebo-controlled trial was performed to investigate the efficacy and safety of rituximab in the treatment of patients with primary SS.

PATIENTS AND METHODS

Study design. This was a prospective, single-center, randomized, double-blind, placebo-controlled study. The study protocol was approved by the institutional review board of the University Medical Center Groningen. All patients provided their written informed consent.

Patients. All patients were age ≥18 years and fulfilled the American–European Consensus Group criteria for primary SS (4). Eligibility criteria were a rate of secretion of stimulated whole saliva of ≥0.15 ml/minute and positivity for autoantibodies (IgM-RF ≥10 IU/ml and anti-SSA and/or anti-SSB autoantibodies). In addition, results from a salivary gland biopsy performed within 12 months before inclusion and showing the characteristic features of SS had to be available (5). During the study, patients were asked to use reliable methods of contraception. Patients with either primary or secondary SS who had been treated previously with other monoclonal antibodies were excluded. Treatment with prednisone and hydroxychloroquine had to be discontinued at least 1 month before baseline, and treatment with methotrexate, cyclophosphamide, cyclosporine, azathioprine, and other disease-modifying antirheumatic drugs had to be discontinued at least 6 months before baseline. Patients were allowed to use artificial tears and artificial saliva, but the regimen had to remain identical during followup. The use of these substitutes had to be stopped 1 day prior to each assessment.

All patients underwent electrocardiography and chest radiography at baseline. Patients with a history of any malignancy or with underlying cardiac, pulmonary, metabolic, renal, or gastrointestinal conditions, chronic or latent infectious diseases, or immune deficiency were excluded.

Drug administration. Twenty patients were treated with intravenous (IV) infusions of 1,000 mg rituximab (Roche, Woerden, The Netherlands) and 10 patients were treated with IV infusions of placebo on days 1 and 15. To minimize side effects (infusion reactions, serum sickness), all patients were pretreated with methylprednisolone (100 mg IV), acetaminophen (1,000 mg orally), and clemastine (2 mg IV), and received 60 mg oral prednisone on days 1 and 2, 30 mg on days 3 and 4, and 15 mg on day 5 after each infusion.

Outcome parameters. *Definition of end points.* The primary end point was defined as a significant improvement in the secretion of stimulated whole saliva (flow rate, in ml/minute) in the rituximab group compared with the placebo group. Secondary end points were measurements of salivary/lacrimal function and immunologic and subjective variables. All variables were assessed at baseline (within 4 weeks before treatment) and at 5, 12, 24, and 48 weeks after treatment.

Determination of salivary gland function. Whole, parotid, and submandibular/sublingual saliva samples were collected in a standardized manner and at a fixed time of the day (in this study, between 1:00 and 4:00 PM), in order to minimize fluctuations related to a circadian rhythm of salivary secretion (6,7) and composition. Glandular saliva was collected from both individual parotid glands by use of Lashley cups, and submandibular/sublingual saliva was collected simultaneously by syringe aspiration from the area with the orifices of the submandibular excretory ducts. Unstimulated saliva was collected in the first 5 minutes, followed by collection of stimulated saliva after the salivary glands had been stimulated for 10 minutes. The salivary glands were stimulated with citric acid solution (2%), applied with a cotton swab to the lateral borders of the tongue every 30 seconds. Flow rates were calculated and the composition of the saliva was analyzed according to the methods described by Burlage et al and Kalk et al (8–10).

Determination of lacrimal gland function. Lacrimal gland function was evaluated by performing the Schirmer's test, the lissamine green test, and breakup time (BUT) (11). Schirmer's test I (without anesthesia) was carried out by placing a filter strip in the lower fornix of the conjunctiva of the eye. The amount of wetting was measured after 5 minutes. The lissamine green test was performed by instillation of 1% lissamine green in both eyes. After 1 or 2 full blinks, the intensity of staining of both medial and lateral bulbar conjunctiva and the cornea was scored, with a maximum score of 9 points (up to 3 points for each section [1 = sparsely scattered, 2 = densely scattered, 3 = confluent]). The BUT is the interval between a complete blink and the appearance of the first randomly distributed dry spots and is assessed by instilling a 1% fluorescein solution in the fornix of both eyes. The patient was asked to blink a few times, after which the intervalin seconds between the last blink and the first break in the tear film was measured.

Laboratory assessments. Laboratory assessments included serum biochemical analyses and determination of the complete blood cell count. Levels of immunoglobulins (IgG, IgA, and IgM) and IgM-RF were measured by nephelometry. Numbers of circulating CD19+, CD4+, and CD8+ T cells were quantified with the use of a FACSCalibur flow cytometer in TruCOUNT tubes (Becton Dickinson, Mountain View, CA). The absolute T cell number was determined by comparing the number of cellular events with that of bead events, analyzed using CellQuest software (Becton Dickinson).

Subjective assessments. Patients completed the Multidimensional Fatigue Inventory (MFI) (12) and the SF-36 health survey (13). In addition, a 100-mm visual analog scale (VAS) was used for rating oral and ocular sicca symptoms.

Extraglandular manifestations. Arthralgia, arthritis, renal involvement, esophageal involvement (confirmed by esophageal scintigraphy), polyneuropathy, Raynaud's phenomenon, fendomyalgia, and vasculitis were defined as extraglandular manifestations. At each visit, extraglandular manifestations were scored as present or not present, according to protocol.

Definition of serum sickness. Serum sickness was defined as the development of fever, lymph node swelling, purpura, myalgia, arthralgia, thrombocytopenia, and proteinuria, as well as a decrease in complement levels. Serum

sickness–like disease was defined as the development of some of these symptoms of serum sickness.

Sample size. Based on a formal sample size calculation, 30 patients were included, of whom 20 were assigned to receive rituximab and 10 to receive placebo. The patients were randomly assigned by staff in the pharmacy department to 1 of the 2 treatment arms in a 2:1 ratio (rituximab:placebo) in blocks of 3, using a random-number generator on a computer. The study investigators (who also provided care and assessed the outcome variables) and patients were blinded to the assigned study medication. The code was revealed to the investigators after followup of all patients was completed. Because of the double-blind design, we assumed a 5% rate of false-positive findings among the patients in the placebo group who displayed clinical signs of serum sickness. This resulted in an obligation to terminate the trial if 2 patients developed clinical signs of serum sickness after the first or second infusion within the first 9 patients, or if 3 patients developed clinical signs of serum sickness after the first or second infusion within the first 29 patients. If, for any reason, the protocol was terminated, patients were not replaced.

Statistical analysis. All data analyses were carried out according to a preestablished plan. To compare treatment effects over time between the 2 treatment groups, repeated-measures analysis of variance was performed. To determine whether an improvement had occurred over time relative to baseline, repeated-measures analysis of covariance was performed to evaluate changes from baseline. Statistical analyses performed on secondary end points were considered to be explorative in nature, and therefore no corrections were made for multiple comparisons. The assumptions on data homogeneity were met. If data were not normally distributed, a log-transformation was performed on the data prior to statistical analysis, or a distribution-free alternative was used.

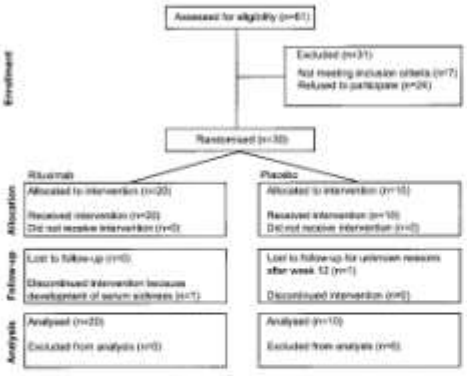

Figure 1. Randomization of patients with primary Sjögren's syndrome to 1 of the 2 treatment groups in the randomized, double-blind, placebo-controlled trial of rituximab. Of a cohort of 300 patients, a preselection of 61 patients was made, based on last available sialometry, IgG, anti-SSA positivity, anti-SSB positivity, and rheumatoid factor data.

Table 1. Baseline characteristics of the patients in the rituximab and placebo treatment groups*

Variable	Placebo (n = 10)	Rituximab (n = 20)
Age, mean ± SD years	43 ± 17	43 ± 11
No. female/no. male	10	19/1
Disease duration, mean ± SD months	67 ± 63	63 ± 50
IgG, mean ± SD gm/liter	21 ± 7	23 ± 8
IgM-RF, mean ± SD IU/ml	221 ± 245	102 ± 78
Anti-Ro/SSA positive	10 (100)	20 (100)
Anti-La/SSB positive	8 (80)	14 (70)
Parotid gland swelling	10 (100)	17 (85)
Whole saliva flow, ml/minute		
Unstimulated	0.06 ± 0.09	0.17 ± 0.19†
Stimulated	0.42 ± 0.26	0.70 ± 0.57
Extraglandular manifestation		
Arthralgia	5 (50)	15 (75)
Arthritis	0 (0)	6 (30)
Renal involvement	0 (0)	2 (10)
Esophageal involvement	1 (10)	0 (0)
Peripheral polyneuropathy	0 (0)	1 (5)
Raynaud's phenomenon	6 (60)	11 (55)
Tendomyalgia	8 (80)	17 (85)
Vasculitis	3 (30)	6 (30)
Thyroid dysfunction	0 (0)	1 (5)
Use of artificial tears	8 (80)	14 (70)
Use of artificial saliva	2 (20)	2 (10)

* Except where indicated otherwise, values are the number (%) of patients. RF = rheumatoid factor.
† $P < 0.05$ versus placebo.

RESULTS

Patient distribution. Between August 2006 and September 2007, 30 patients were randomly assigned to a treatment group (Figure 1). The baseline characteristics of the patients are summarized in Table 1. Six patients were taking medication that had to be discontinued before study inclusion, in accordance with the inclusion criteria.

Efficacy (Table 2). *Salivary gland function.* The stimulated whole saliva flow rate (the primary end point) (Figure 2A) significantly improved in the rituximab group ($P = 0.018$ at week 5 and $P = 0.004$ at week 12, versus baseline), while in the placebo group these values significantly decreased from baseline, which is consistent with the natural progression of the disease. A significant difference in the mean change from baseline to week 12 in the stimulated whole saliva flow rate was found between the groups ($P = 0.038$). The unstimulated whole saliva flow rate (Figure 2B) and the submandibular/sublingual flow rate (results not shown) also significantly increased from baseline in the rituximab group.

Lacrimal gland function. The LG test showed significant improvement in lacrimal gland function in the rituximab group from baseline to weeks 5–48. However,

Table 2. Results of laboratory, functional, and subjective assessments in the rituximab and placebo treatment groups*

Variable	Baseline		Week 5		Week 12		Week 24		Week 36		Week 48	
	Placebo	Rituximab	Placebo	Rituximab	Placebo	Rituximab	Placebo	Rituximab	Placebo	Rituximab	Placebo	Rituximab
Whole saliva flow, ml/minute†												
Unstimulated	0.06 ± 0.09 (0.05)	0.17 ± 0.19 (0.08)	0.09 ± 0.07 (0.06)	0.24 ± 0.22 (0.20)‡	0.05 ± 0.05 (0.04)	0.23 ± 0.22 (0.19)‡	0.08 ± 0.06 (0.09)	0.22 ± 0.25 (0.14)	0.07 ± 0.09 (0.02)	0.16 ± 0.15 (0.11)	0.05 ± 0.04 (0.04)‡	0.18 ± 0.18 (0.13)
Stimulated	0.82 ± 0.26 (0.36)	0.70 ± 0.57 (0.47)	0.83 ± 0.24 (0.37)	0.64 ± 0.71 (0.48)‡	0.28 ± 0.17 (0.25)‡	0.67 ± 0.87 (0.56)‡§	0.36 ± 0.28 (0.24)	0.78 ± 0.69 (0.52)	0.29 ± 0.18 (0.26)‡	0.64 ± 0.58 (0.44)	0.28 ± 0.23 (0.22)‡	0.66 ± 0.71 (0.42)
Lacrimal gland function†												
Schirmer's test, mm/5 minutes	7 ± 9 (3)	11 ± 11 (7)	7 ± 11 (4)	10 ± 9 (10)	6 ± 5 (5)	11 ± 10 (11)	8 ± 8 (6)	12 ± 12 (5)	7 ± 7 (5)	11 ± 10 (7)	5 ± 5 (6)‡	10 ± 11 (7)
Lissamine green test	4 ± 1 (4)	5 ± 2 (4)	5 ± 1 (5)	3 ± 2 (3)‡	4 ± 2 (4)	3 ± 2 (3)‡	4 ± 2 (4)	2 ± 2 (2)‡	4 ± 2 (4)	2 ± 2 (2)‡	4 ± 2 (4)	2 ± 3 (1)‡
Tear breakup time, seconds	3 ± 2 (3)	6 ± 2 (6)	3 ± 1 (3)	6 ± 3 (6)	3 ± 2 (3)	5 ± 3 (5)	5 ± 2 (6)‡	6 ± 3 (7)	5 ± 3 (5)‡	7 ± 3 (6)‡	4 ± 3 (4)	6 ± 3 (6)
B cells, 10^9/liter†	0.27 ± 0.12 (0.26)	0.23 ± 0.17 (0.18)	0.20 ± 0.09 (0.17)‡	0.00 ± 0.00 (0.00)‡§	0.25 ± 0.10 (0.27)‡	0.01 ± 0.03 (0.00)‡§	0.28 ± 0.11 (0.26)‡	0.05 ± 0.06 (0.03)‡§	0.26 ± 0.12 (0.31)‡	0.10 ± 0.06 (0.08)‡§	0.33 ± 0.13 (0.37)‡	0.17 ± 0.10 (0.15)‡§
IgM RF, IU/ml†	221 ± 245 (106)	102 ± 79 (83)	162 ± 175 (99)‡	55 ± 36 (51)‡	156 ± 138 (102)‡	44 ± 30 (39)‡§	226 ± 260 (133)‡	45 ± 34 (32)‡§	255 ± 256 (159)‡	71 ± 68 (59)‡§	225 ± 199 (128)‡	103 ± 103 (72)‡
MFI, general fatigue	14 ± 5 (17)	16 ± 4 (18)‡	11 ± 5 (12)‡	15 ± 4 (16)‡	13 ± 5 (14)‡	15 ± 4 (13)‡	12 ± 5 (12)‡	15 ± 4 (12)‡	14 ± 4 (14)‡	14 ± 4 (14)‡	14 ± 6 (17)‡	15 ± 4 (16)‡
SF-36 total score	64 ± 17 (65)‡	52 ± 20 (53)‡	76 ± 17 (70)‡	56 ± 18 (52)‡	67 ± 15 (71)‡	63 ± 15 (65)‡	72 ± 16 (82)‡	67 ± 16 (70)‡	63 ± 16 (65)‡	60 ± 17 (64)‡	62 ± 17 (62)‡	55 ± 18 (55)‡
VAS score, oral dryness	59 ± 28 (62)‡	55 ± 28 (61)‡	59 ± 28 (53)‡	47 ± 27 (53)‡	53 ± 30 (60)‡	40 ± 27 (40)‡	64 ± 27 (74)‡	34 ± 27 (46)‡	68 ± 26 (79)‡	51 ± 28 (61)‡	69 ± 25 (76)‡	50 ± 28 (53)‡
VAS score, ocular dryness	65 ± 27 (63)‡	59 ± 29 (68)‡	55 ± 28 (52)‡	49 ± 28 (51)‡	61 ± 25 (54)‡	48 ± 29 (47)‡	68 ± 24 (74)‡	41 ± 28 (43)‡	70 ± 27 (72)‡	46 ± 27 (52)‡§	76 ± 19 (80)‡	46 ± 28 (55)‡§

* Values are the mean ± SD (median). Due to missing data, the differences between mean values in this table differ slightly from the means of differences shown in Figure 2. RF = rheumatoid factor; MFI = Multidimensional Fatigue Inventory; SF-36 = Short Form 36; VAS = visual analog scale.

† Data are not normally distributed.

‡ $P < 0.05$ versus baseline in the same treatment group, by analysis of covariance.

§ $P < 0.05$ versus change from baseline in the placebo group, by analysis of variance.

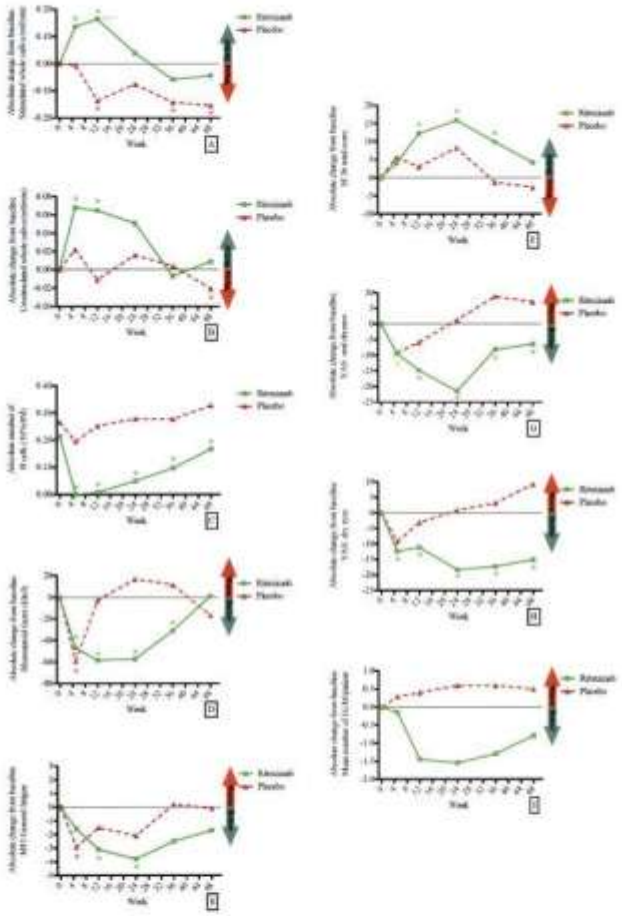

Figure 2. Mean values of absolute change from baseline (**A**, **B**, and **D**–**I**) and mean absolute number of B cells (**C**) in the rituximab group compared with the placebo group. The primary end point was **A**, the rate of secretion of stimulated whole saliva, while the secondary end points were **B**, the rate of secretion of unstimulated whole saliva, **C**, absolute number of B cells, **D**, rheumatoid factor levels, **E**, Multidimensional Fatigue Inventory (MFI) score for general fatigue, **F**, Short Form 36 (SF-36) total score, **G**, visual analog scale (VAS) score for oral dryness, **H**, VAS score for ocular dryness, and **I**, mean number of extraglandular manifestations (EGM) per patient. * = $P < 0.05$ versus baseline.

the Schirmer's test and BUT test revealed no significant changes in lacrimal gland function in either group.

Changes in laboratory variables. B cells were completely depleted after the first infusion in patients treated with rituximab (Figure 2C). In contrast, no significant changes in the mean absolute number of B cells were found in the placebo group. In the patient who developed serum sickness (see data on safety assess-

ments below), who received only 1 infusion of rituximab, B cells reappeared within 12 weeks after treatment. In the other 19 rituximab-treated patients, B cells returned within 24–48 weeks after treatment, although B cell levels still had not returned to baseline values by week 48. Significant differences in the mean change in absolute B cell count from baseline to weeks 5, 12, 24, 36, and 48 were found between the groups (each $P < 0.05$). No significant changes were found in the levels of CD4+ and CD8+ T cells in either the rituximab group or placebo group.

Levels of RF (Figure 2D) decreased significantly in the rituximab group over week 5 to week 36, whereas in the placebo group, the RF levels decreased significantly only at week 5. Significant differences in the mean change in RF levels from baseline between the groups were found at weeks 12, 24, and 36 (each $P < 0.05$). The same patterns of change were found for the levels of IgG, IgM, and IgA in each group (results not shown).

Changes in subjective measurements. The MFI and SF-36 scores showed the strongest improvements in the rituximab group (Figures 2E and F). Compared with that in the placebo group, patients receiving rituximab showed a significant change in the MFI score, showing decreased scores for reduced activity from baseline to week 36 ($P = 0.023$) and for reduced motivation from baseline to week 12 ($P = 0.039$). In addition, in patients receiving rituximab, there was significant improvement in the SF-36 score for vitality from baseline to week 36 ($P = 0.013$). Moreover, all VAS scores for oral and ocular sicca symptoms improved in the rituximab group (Table 2 and Figures 2G and H), whereas VAS scores in the placebo group only showed a significant improvement at week 5. Significant differences in the mean change in VAS scores from baseline were observed between the groups, in that patients receiving rituximab reported improvement in the ratings for dry mouth during the night at weeks 24, 36, and 48 and in the ratings for dry eyes at weeks 36 and 48 in the rituximab group (each $P < 0.05$).

Extraglandular manifestations. At baseline, there were no differences in the number of extraglandular manifestations between the rituximab group and placebo group (Figure 2I). The number of reported extraglandular manifestations (number reported as present) significantly decreased in the rituximab group compared with the placebo group for tendomyalgia at weeks 12 and 36 ($P = 0.029$) and for vasculitis at week 24 ($P = 0.030$). In addition, there was a strong tendency toward a significant decrease in the number of reported symptoms of Raynaud's phenomenon ($P = 0.057$), tendomyalgia ($P = 0.074$), and arthralgia ($P = 0.058$) from baseline to week 24 in patients receiving rituximab. Six patients in the rituximab group had symptoms of arthritis at baseline; this resolved in 4 patients during followup. In the placebo group, no patients had symptoms of arthritis at baseline; however, 3 patients developed symptoms during followup. One patient with decreased thyroid function before rituximab treatment showed a normalization of thyroid function without additional thyrostatic supplementation. Renal function remained stable during followup (2 patients had renal tubular acidosis, and both were treated with rituximab). Clinical symptoms of polyneuropathy (in 1 patient in the rituximab group) improved after 12 weeks of followup.

Table 3. Adverse events observed in patients following treatment with rituximab as compared with placebo*

Event	Placebo (n = 10)	Rituximab (n = 20)
Early infusion reaction	0	2 (10)
Late infusion reaction	0	2 (10)
Serum sickness	0	1 (5)
Infections within 2 weeks after infusion		
Upper airway infection	0	1 (5)
Parvovirus	0	1 (5)
Infections during 48 weeks of followup		
Otitis media	0	2 (10)
Upper airway infection	4 (40)	4 (20)
Recurrence of ocular toxoplasmosis	0	1 (5)
Parotid gland infection	0	3 (15)
Recurrence of herpes zoster	1 (10)	0
Epstein-Barr virus	1 (10)	0
Rubella	1 (10)	0

* Values are the number (%) of patients.

Safety (Table 3). *Serum sickness.* One female patient with diabetes developed a mild serum sickness–like disease, which was identified 14 days after the first infusion of rituximab. The patient developed fever, purpura on both legs, and arthralgia, and she was admitted to the hospital in order to control her serum glucose levels during IV administration of corticosteroids and nonsteroidal antiinflammatory drugs. She recovered completely within a few days, without developing human antichimeric antibodies. The second infusion of rituximab was not administered. This patient had not been treated with any immunosuppressive drug previously. None of the 6 patients who had discontinued immunosuppressive drugs 1–6 months prior to rituximab treatment developed serum sickness–like disease.

Infections. A total of 12 infections were reported by 11 patients in the rituximab group, while 4 patients in the placebo group reported a total of 7 infections. The rates of infection were 76 and 65 events per 100 patient-years for the placebo and rituximab groups, respectively.

None of the infections required hospitalization. No opportunistic infections were observed.

DISCUSSION

This study showed that rituximab-induced B cell depletion can be considered an effective and safe treatment strategy for patients with primary SS. B cell depletion resulted in improvement of objective and subjective parameters of disease activity in patients with primary SS for at least 6–9 months. Among the end points, salivary gland function improved, fatigue diminished, and the number of extraglandular manifestations was reduced.

Rituximab has already been shown to be a safe and effective treatment for rheumatoid arthritis (RA), as shown by a decrease in disease activity, diminished radiologic progression of the disease, and improved quality of life in patients with RA (14–16). Previously, the utility of rituximab for the treatment of SS had only been investigated in a few open-label, phase II studies and in 1 randomized, double-blind, placebo-controlled study. Results from open-label studies, in terms of objective and subjective variables, were promising (2,3), as was the improvement of systemic features (17). Although the duration of the treatment effect differed between the trials, in all trials a significant effect occurred 12–24 weeks after treatment. In a previous randomized, double-blind, placebo-controlled study of rituximab treatment of SS, a significant improvement in fatigue (the primary end point) was noted as compared with the values at baseline in the rituximab group, but there were no significant changes in the secondary end points assessing glandular manifestations (unstimulated salivary flow rate and Schirmer's test results) (18). Moreover, the study by Dass et al (18) used an objective eye test for lacrimal gland function that was less accurate (the Schirmer's test); the rose bengal score and LG test are considered to be more accurate (11). This fact, together with the small number of patients included in that trial (8 receiving rituximab, 9 receiving placebo), might explain the lack of significant improvement in glandular manifestations following rituximab treatment.

In our trial, most significant improvements in the end points associated with rituximab treatment were observed between 12 weeks and 36 weeks following treatment. In contrast, improvement of most of the variables observed in patients in the placebo group occurred 5 weeks after the first infusion. We hypothesize that the improvements observed after placebo treatment were related to the treatment with prednisolone, which had been administered before and during the days after the infusions, although data are inconclusive regarding the effect of prednisolone on SS symptoms. Although results of a previous study indicated a significant increase in whole saliva flow during the use of low-dose prednisolone (19), other studies noted no significant improvement in glandular function (20,21).

The flow rate of stimulated whole saliva provides a general indication of overall salivary glandular function, which is an important outcome in a disease that specifically affects the salivary glands. Pijpe et al (3) reported the occurrence of a significant increase in the stimulated whole saliva flow rate in rituximab-treated patients with primary SS whose stimulated salivary flow rate was >0.10 ml/minute at baseline. These patients also showed significant improvement in such subjective parameters as mouth dryness, arthralgia, physical functioning, vitality, and most domains of the MFI. In other words, patients with some residual secretory potential may benefit the most from rituximab treatment. The secretory potential at baseline might even be used to identify those patients who would be considered to be a good responder to rituximab treatment. Therefore, the stimulated whole saliva flow rate was chosen as the primary end point of our study. As a cutoff value, a stimulated whole saliva flow rate of ≥0.15 ml/minute was chosen, since this is a flow rate that discriminates patients showing increasing disease activity (e.g., progressive loss of secretory function) and patients with end-stage primary SS (21). In our study, we observed an increase in salivary flow in the rituximab group that exceeded the intrapatient variability observed for repeated collections of saliva (8). This increase was also reflected in the improvements in subjective scores for dry mouth, which indicates that these changes were clinically meaningful in the patients. The nonsignificant baseline difference between the groups for the stimulated whole saliva flow rate was caused by high salivary flow rates in a few patients before inclusion. All patients in the study were required to have a stimulated whole saliva flow of ≥0.15 ml/minute. This meant that all patients had a clinically relevant functional secretory salivary gland capacity. Our pilot study revealed that no relevant improvement in salivary gland function can be expected in patients with little or no secretory potential at baseline.

In clinical trials of rituximab in patients with RA, the number of reported (serious) infections and infusion reactions is within the range expected for patients with RA treated with biologic agents. Therefore, the risk: benefit ratio is considered to be good regarding rituximab treatment of RA (22). In clinical trials of rituximab

treatment for other autoimmune diseases (including SS), the reported numbers of infusion reactions and infections have varied widely; this is possibly due to variability in how these adverse events are defined or to the small numbers of patients. The incidence of infusion reactions and infections reported for the rituximab group in this trial was largely comparable with that in the placebo group, and was lower or within the same range as that reported previously (23). Moreover, the rate of infections per 100 patient-years was lower compared with the previously reported rate in RA patients treated with rituximab. This might be explained by the fact that our patients did not have any other immunosuppressive therapy (24).

When compared with patients with lymphoma, patients with RA, and patients with systemic lupus erythematosus (SLE) treated with rituximab, patients with primary SS treated with rituximab develop serum sickness–like disease more frequently (6–27%) (25). A therapy-related explanation for this phenomenon might be that patients with RA and those with SLE usually receive or have received higher doses of steroids and/or other immunosuppressive drugs, in addition to rituximab, whereas our patients with primary SS received no other medication, except a 5-day period of steroids after IV administration of rituximab. Another therapy-related explanation is that patients with RA and those with SLE often have been exposed to intensive immunosuppressive regimens, including treatment with biologic agents, before they undergo treatment with rituximab, whereas our patients with primary SS were far more likely to have never taken such medications at the time of rituximab treatment. The higher susceptibility for serum sickness could also be inherent to the disease itself. The patients with primary SS in this trial, as well as in our pilot trial (3), who developed serum sickness were more likely to have an active, early, and progressive form of SS. It is possible that such patients with primary SS are more prone to develop serum sickness. Furthermore, hypergammaglobulinemia is common in primary SS, which could make these patients prone to the development and deposition of immune complexes and, thus, to serum sickness–like disease (18).

Because of the higher risk of developing serum sickness–like disease in patients with SS, we decided to increase the steroid dose. Only 1 patient in the current study developed serum sickness–like disease (5%), which is considerably lower than the incidence reported in our open-label study (27%) (3). Based on these findings, we would recommend administering 100 mg methylprednisolone immediately prior to each infusion of rituximab. The oral regimen of prednisolone in the days following each infusion is a point of interest and should be explored in future trials. The administration of higher doses of prednisolone in the days following infusion, such as is performed during lymphoma treatment, should also be considered.

This study indicates that rituximab treatment could be effective for patients who have active primary SS and remaining salivary gland secretory potential, as well as for primary SS patients with extraglandular manifestations. Future trials of rituximab treatment for patients with primary SS are warranted, in which larger groups of patients should be included and less-strict inclusion criteria (e.g., no restriction to those with salivary gland function ≥0.15 ml/minute and autoantibody positivity) should be used, in order to be able to extrapolate the results to a larger group of patients with primary SS. In addition to the defined inclusion criteria, attention should be given to the criteria used for response to treatment. Activity scores for primary SS have now been developed and need validation. These scores should be included in the response criteria to be used in future trials.

Based on the promising results of this study and our prior study on retreatment with rituximab, which resulted in a beneficial effect comparable with that of the first treatment with this biologic agent (26), a maintenance therapy with rituximab infusions every 6–9 months may be a reasonable approach. Advantages of maintenance therapy might be a reduction or even arrest of disease progression and improvement of quality of life for a long period. This improvement will be a great achievement in patients with SS, since SS has a large impact on health-related quality of life, employment, and disability (1). A threat might be the long-term side effects (thus-far unknown) of repeated B cell depletion. The timing of retreatment could be based on return of symptoms; however, retreatment just before return of symptoms would even be better.

In conclusion, the results of this study indicate that rituximab could be an effective and safe treatment strategy for patients with primary SS. B cell depletion resulted in improvement of the primary end point, the rate of stimulated whole saliva secretion. Explorative analyses also showed improvements, of at least 6–9 months' duration, in the objective and subjective secondary end points of disease activity. Since primary SS has a great impact on health-related quality of life, employment, and disability (1), it is worthwhile to further explore the role of rituximab in a large-size, randomized, controlled trial.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Vissink had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Meijer, Vissink, Spijkervet, Brouwer, Kallenberg, Bootsma.

Acquisition of data. Meijer, Meiners, Spijkervet, Abdulahad, Kamminga, Brouwer, Bootsma.

Analysis and interpretation of data. Meijer, Meiners, Vissink, Abdulahad, Kallenberg, Bootsma.

ROLE OF THE STUDY SPONSOR

This trial was an investigator-driven study that was financially supported by Roche (Woerden, The Netherlands), which also supplied the study medication. There was no involvement of the study sponsor in the study design, patient recruitment, data collection, analysis and interpretation of the data, or writing of the report. Statistical analyses were performed by staff at the statistical department of Xendo Drug Development BV (Groningen, The Netherlands), which is an independent contract research organization. Medical writing support was provided by staff at Adelphi Communications (supported by F. Hoffmann-La Roche, Ltd.) during the final preparation of the article.

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Treatment of Primary Sjögren Syndrome With Rituximab

A Randomized Trial

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Background: Primary Sjögren syndrome (pSS) is an autoimmune disorder characterized by ocular and oral dryness or systemic manifestations.

Objective: To evaluate efficacy and harms of rituximab in adults with recent-onset or systemic pSS.

Design: Randomized, placebo-controlled, parallel-group trial conducted between March 2008 and January 2011. Study personnel (except pharmacists), investigators, and patients were blinded to treatment group. (ClinicalTrials.gov: NCT00740948)

Setting: 14 university hospitals in France.

Patients: 120 patients with scores of 50 mm or greater on at least 2 of 4 visual analogue scales (VASs) (global disease, pain, fatigue, and dryness) and recent-onset (<10 years) biologically active or systemic pSS.

Intervention: Randomization (1:1 ratio) to rituximab (1 g at weeks 0 and 2) or placebo.

Measurements: Primary end point was improvement of at least 30 mm in 2 of 4 VASs by week 24.

Results: No significant difference between groups in the primary end point was found (difference, 1.0% [95% CI, −16.7% to 18.7%]). The proportion of patients with at least 30-mm decreases in at least two of the four VAS scores was higher in the rituximab group at week 6 (22.4% vs. 9.1%; $P = 0.036$). An improvement of at least 30 mm in VAS fatigue score was more common with rituximab at weeks 6 ($P < 0.001$) and 16 ($P = 0.012$), and improvement in fatigue from baseline to week 24 was greater with rituximab. Adverse events were similar between groups except for a higher rate of infusion reactions with rituximab.

Limitation: Low disease activity at baseline and a primary outcome that may have been insensitive to detect clinically important changes.

Conclusion: Rituximab did not alleviate symptoms or disease activity in patients with pSS at week 24, although it alleviated some symptoms at earlier time points.

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Primary Sjögren syndrome (pSS) is a chronic autoimmune disorder characterized by dryness of the eyes and mouth and systemic involvement in up to 50% of cases (1). Histopathology shows lymphocytic infiltration and destruction of the lachrymal and salivary glands (2). To date, no systemic treatment has been proved to significantly affect the course of pSS (3), but clinicians may prescribe hydroxychloroquine to patients having fatigue or arthralgia and corticosteroids, methotrexate, or immunosuppressants to patients with systemic involvement. Because mounting evidence points to a central pathophysiologic role for B cells (4–7), B-cell depletion is being evaluated as a treatment of pSS (8–11).

The most widely studied target for achieving B-cell depletion is the CD20 antigen, a transmembrane protein found on pre-B and mature B cells. It is neither shed from the cell surface nor internalized on antibody binding (12–14). In open-label studies, the anti-CD20 antibody rituximab had a good safety profile, induced rapid B-cell depletion in blood and salivary glands, and seemed beneficial in early active pSS and in pSS with active extraglandular involvement (8, 9, 15). Two small, double-blind, randomized trials have been published (10, 11). The first included 18 patients and suggested an effect on the visual analogue scale (VAS) fatigue score after 6 months, although the primary end point, a 20% or greater decrease in the VAS fatigue score, was not achieved (10). The second trial included 30 patients with recent active pSS and showed improvements in the VAS dryness score and stimulated total salivary flow rate after 6 months (11).

The purpose of the randomized, placebo-controlled TEARS (Tolerance and Efficacy of Rituximab in Primary Sjögren's Syndrome) trial reported here was to evaluate the efficacy and adverse effects of rituximab in pSS.

METHODS

Design Overview

This randomized, placebo-controlled, parallel-group trial evaluated global disease, pain, fatigue, and dryness. French rheumatologists and internists recruited the pa-

See also:

Print
Summary for Patients 1-11

Web-Only
Supplement

Context

Few trials have examined treatments for primary Sjögren syndrome (pSS).

Contribution

This multicenter, double-blind, placebo-controlled, randomized trial found that rituximab (given in 2 infusions over 2 weeks) alleviated some symptoms at week 6 but did not alleviate symptoms or improve global activity score at month 6 in adults with recent-onset or systemic pSS. More infusion reactions occurred with rituximab than placebo.

Caution

Outcome measurements may have been insensitive for detecting improvement.

Implication

Rituximab infusions did not produce sustained or substantial alleviation of symptoms or improvement in disease activity in adults with recent-onset or systemic pSS.

—The Editors

tients between 6 March 2008 and 5 January 2011. Patients were randomly assigned in a 1:1 ratio to blinded treatment with intravenous infusions of rituximab (1 g) or placebo at weeks 0 and 2. All study personnel, investigators, and patients remained blinded to the treatment group throughout the study. This study was approved by the appropriate ethics committee (CPP Ouest VI), and all patients gave written informed consent before study enrollment. The protocol was registered on ClinicalTrials.gov (NCT00740948).

Setting and Participants

Patients were recruited at 14 university hospitals in France if they fulfilled the American–European Consensus Group criteria for pSS (16) and had active disease, defined as scores of at least 50 mm on at least 2 of 4 VASs (scores range from 0 [none] to 100 mm [worst]) for global disease, pain, fatigue, and dryness. Additional requirements were onset of pSS symptoms (first visit for any sign) in the past 10 years and biologically active pSS (defined as autoantibodies [anti-Ro/SSA antibodies or rheumatoid factor], cryoglobulinemia, hypergammaglobulinemia, β_2-microglobulin elevation, or hypocomplementemia) or systemic pSS with at least 1 extraglandular manifestation or current parotid gland enlargement. The other inclusion criteria were informed consent, being aged 18 to 80 years, stable nonsteroidal anti-inflammatory drug regimen, no use of immunosuppressive agents for at least 4 weeks before inclusion, and use of an effective contraceptive method for patients able to conceive. Exclusion criteria were secondary Sjögren syndrome; cytotoxic drug therapy in the past 4 months; severe renal or hematologic failure; history of cancer, hepatitis B or C, HIV infection, tuberculosis, severe diabetes, or any other chronic disease; evidence of infection; history of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies; and an inability to understand the study protocol.

Randomization and Interventions

Randomization was stratified by site. A computer-generated random allocation sequence was prepared by our statistics department in Brest, France. The infusions were prepared after a telephone call to the statistics department by pharmacists who were not involved in any other study procedure and were instructed not to disclose the treatment group to the investigators. All patients received the same volume, but the infusion contained the solvent only (normal saline or 5% glucose) in the placebo group and the solvent plus rituximab in the rituximab group. Before each rituximab or placebo infusion, the patients received 100 mg of methylprednisolone intravenously and 500 mg of acetaminophen orally.

Outcomes and Follow-up

Efficacy was evaluated at weeks 6, 16, and 24. The primary outcome, chosen a priori on the basis of expert opinion, was a 30-mm or greater improvement at week 24 versus baseline on at least 2 of the 4 VAS scores.

Secondary outcomes included variations from baseline in the individual VAS scores at weeks 6 and 16; disease activity, systemic manifestations, and treatment activity assessed by the investigator as present or absent and by using both a physician VAS for disease activity and the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI), a clinical index that is designed to measure disease activity in patients with pSS (12 domains with a total score ranging from 2 to 47) (17, 18); basal salivary flow rate; Schirmer test and van Bijsterveld scores and Chisholm grade (19, 20); C-reactive protein level and erythrocyte sedimentation rate; rheumatoid factor; antinuclear antibodies; serum IgG, IgA, and IgM levels; serum complement; cryoglobulinemia; and serum level of B-cell–activating factor (BAFF) (21).

Clinicians collected open-ended adverse events and assessed severity and potential causality at each visit from baseline to week 24. At study completion, the chief investigator categorized the adverse events according to the Medical Dictionary for Regulatory Activities, which is required by European regulations. We used Lower-Level Terms in the System Organ Class system.

Statistical Analysis

Our power calculation was based on our primary end point assessed at week 24. To detect a difference of 30 percentage points between groups in the proportion of patients achieving the primary end point, with a 2-sided α of 0.05 and 80% power, we needed 49 patients per group. We planned to enroll 120 patients to allow for withdrawals and missing data.

All randomly assigned patients who did not withdraw before the first study-drug infusion were included in the

efficacy analyses. They were analyzed in the group to which they had been randomly assigned, even when a protocol deviation was reported (intention-to-treat principle). We used a fully conditional specification method to do multiple imputation and to handle missing data, which were assumed to be missing at random. We used the MICE function in R, version 2.14 (R Foundation for Statistical Computing, Vienna, Austria), to generate 20 imputed data sets. The initial data set to impute contained all outcomes of interest, baseline characteristics, and center and random assignments. To build the imputation model, we used the quickpred function in R to include all predictors with an absolute correlation of at least 0.2 with the target or the response indicator. Study center and treatment group were forced to be included in the imputation model. Continuous variables that were clearly nonnormal were transformed before imputation then back-transformed to create the final imputed data set.

We analyzed the primary outcome at week 24 using a generalized linear model with binomial distribution, identity link, and exchangeable correlation structure to account for study center. Although identity is not the usual link for a binary response, it can be used in the present situation (22, 23) to estimate a risk difference with the CI, as recommended by the CONSORT (Consolidated Standards of Reporting Trials) statement. Secondary outcomes were analyzed by using the same statistical method, except a normal distribution was used for continuous data. All efficacy analyses were first done for each week by using the imputed data, except for the serum BAFF level, which was not collected in all study centers and was analyzed by using the observed data. Reductions in BAFF levels were compared between the rituximab and placebo groups by using the Wilcoxon test.

For the 4 VASs used to define the primary end point, longitudinal analyses were then done on the observed data by using a mixed model. In these analyses, we used treatment group, visit, and the visit–treatment group interaction term as independent variables; study center as a random-effects factor; a compound symmetry covariance structure to account for repeated measures among visits; and age, sex, baseline antibody values, and recent-onset or systemic pSS information as covariates.

We used SAS, version 9.3 (SAS Institute, Cary, North Carolina), for all analyses except for multiple imputation, for which we used R, version 2.14. P values less than 0.05 were considered statistically significant. We used the MICE function in R to generate imputed data sets, PROC MIANALYZE in SAS to obtain pooled estimates, and PROC GENMOD and PROC MIXED in SAS to build generalized linear models and mixed models and to make statistical inferences based on t tests or F tests. Graphical representations of longitudinal data were obtained from estimates given by the LSMEANS statement of the MIXED procedure in SAS. This allowed a representation of the course of VAS values when identical baseline characteristics that were taken to be equal to the mean values were assumed.

We did sensitivity analyses to check the robustness of the results according to the method used to handle missing data (**Appendix Table 1**, available at www.annals.org). First, analyses were done only on available data, assuming that data were missing completely at random. Second, the missing data for pain, fatigue, dryness, and global VAS scores (≥30-mm decrease) at weeks 6, 16, and 24 were imputed as a failure to investigate the effects on statistical results if the data would have been more likely to be missing in patients without improvements of at least 30 mm. Models used for sensitivity analyses were the same as for the primary analyses (for example, the generalized linear model).

Role of the Funding Source

The Programme Hospitalier de Recherche Clinique 2010 (the French public research funding agency) funded this study. Rituximab was donated free of charge by Roche (Boulogne Billancourt, France). Neither the funding source nor Roche had any role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, revision, or approval of the manuscript.

RESULTS

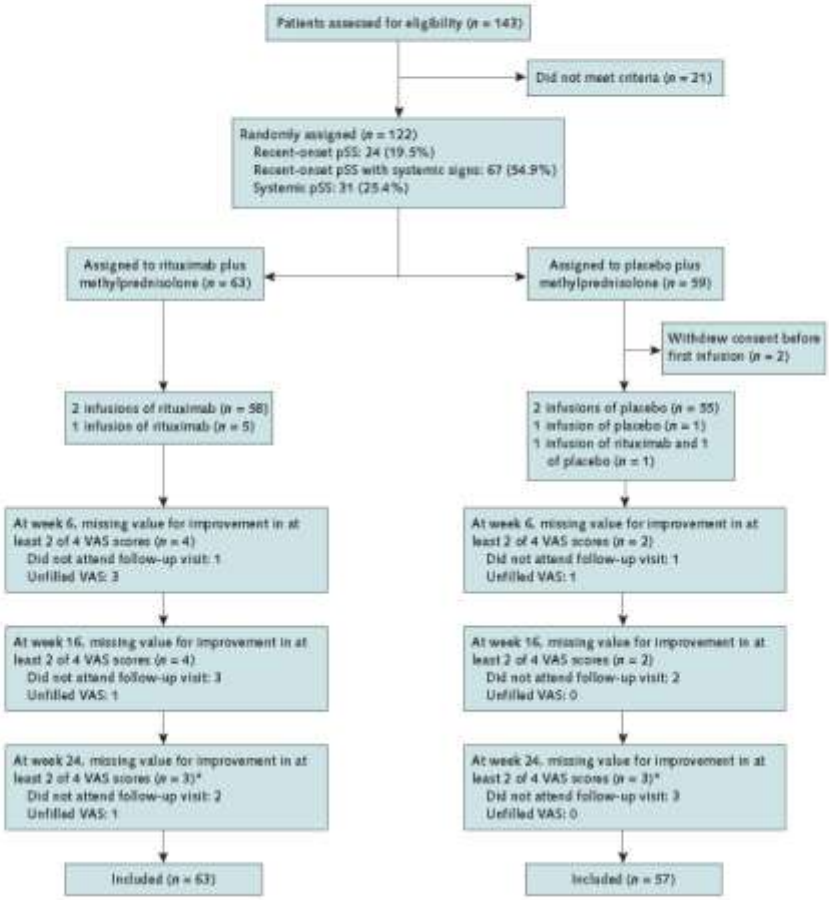
Patient Characteristics

We randomly assigned 122 patients with pSS, including 24 with recent-onset pSS, 31 with systemic pSS, and 67 with both. Among them, 120 received at least 1 infusion and were included in the analysis (**Figure 1**). Five patients in the rituximab group and 1 in the placebo group did not receive the second infusion because of adverse events. Baseline characteristics were similar between groups (**Table 1**). Most patients were women; 97 (80.8%) had anti-Ro/SSA or anti-La/SSB antibodies, and 105 (87.5%) had abnormal salivary gland biopsy results (focus score ≥1). The mean ESSDAI score was 10.1 (SD, 6.8). Mean global disease VAS scores in the placebo and rituximab groups were 69.4 (SD, 17.0) and 68.9 (SD, 15.6), respectively. The primary co-interventions that patients received were steroid therapy (34 patients), methotrexate (17 patients), and local therapy, but their variations were considered negligible by the investigators during follow-up.

Efficacy

At weeks 6, 16, and 24, the percentages of patients with decreases of at least 30 mm in at least 2 of the 4 VAS scores were 9.1%, 17.0%, and 22.0%, respectively, in the placebo group and 22.4%, 26.3%, and 23.0%, respectively, in the rituximab group. The percentage was larger in the rituximab group only at week 6 (difference, 13.3 percentage points [95% CI, 0.8 to 25.8 percentage points]; $P = 0.036$) (**Table 2**). For the primary outcome (week 24), the difference (1.0 percentage point [CI, −16.7 to

Figure 1. **Study flow diagram.**



pSS = primary Sjögren syndrome; VAS = visual analogue scale.
* Primary outcome is improvement in ≥2 of 4 VAS scores at week 24.

18.7 percentage points]) was not significant. The sensitivity analysis confirmed the robustness of our results (**Supplement**, available at www.annals.org).

Table 2 reports the secondary efficacy outcomes (proportion of patients having an improvement and magnitude of the improvement). A 30-mm decrease in the VAS fatigue score was more common with rituximab than placebo at week 6 (absolute difference, 26.6 percentage points [CI, 15.7 to 37.5 percentage points]; $P < 0.001$) and week 16 (absolute difference, 18.3 percentage points [CI, 4.1 to 32.6 percentage points]; $P = 0.012$). The difference in the percentage of patients with a VAS dryness score that decreased by at least 30 mm at any of the time points was not clinically significant between groups (absolute differences of 8.0 percentage points [CI, −3.7 to 19.7 percentage points], 7.5 percentage points [CI, −5.4 to 20.4 percentage points], and 12.4 percentage points [CI, −3.0 to 27.8 percentage points] at weeks 6, 16, and 24, respectively).

Figure 2 shows the course of VAS scores from baseline to week 24 in the rituximab and placebo groups after adjustment for baseline characteristics, as well as results of longitudinal data analyses. The mean decrease in the VAS

fatigue score was larger with rituximab than placebo at weeks 6, 16, and 24. Pain was not alleviated by rituximab at any of the evaluation time points. The **Supplement** shows data plots for each patient over time by baseline value (≤50 or >50 mm).

At week 6, more patients in the rituximab group than the placebo group had improvements in physician-assessed disease activity (absolute difference, 19.1 percentage points [CI, 4.4 to 33.7 percentage points]), treatment efficacy (absolute difference, 21.0 percentage points [CI, 9.3 to 32.7 percentage points]), and global VAS score (mean difference, 8.4 mm [CI, 4.2 to 12.5 mm]); this was not the case at weeks 16 or 24 (**Table 2**). The decrease in ESSDAI score was not larger with rituximab than placebo at week 6 (mean difference, −0.3 [CI, −1.2 to 0.7]), week 16 (mean difference, −0.3 [CI, −1.7 to 1.0]), or week 24 (mean difference, −0.5 [CI, −2.3 to 1.3]), including for the biological domain (**Supplement**). The proportions of patients with systemic pSS who had resolution of parotid gland enlargement or joint involvement were not higher with rituximab than placebo (40 of 54 patients [74.1%] in the placebo group and 47 of 61 patients [77%] in the rituximab group had an ESSDAI glandular item scored 0 at week 24 [**Appendix Table 2**, available at www.annals.org]).

At week 24, serum IgG, IgA, IgM, and β_2-microglobulin levels were more improved with rituximab (IgG difference, 1.2 g/L [CI, 0.4 to 2.0 g/L]; $P = 0.003$; IgA difference, 0.5 g/L [CI, 0.0 to 1.1 g/L]; $P = 0.047$; IgM difference, 0.3 g/L [CI, 0.2 to 0.4 g/L]; $P < 0.001$; β_2-microglobulin difference, 1.6 g/L $\times 10^{-4}$ [CI, 0.5 to 2.8 g/L $\times 10^{-4}$]; $P = 0.004$). The serum BAFF levels at baseline and week 24 were 4.63 ng/mL (SD, 12.42 ng/mL) and 2.43 ng/mL (SD, 7.31 ng/mL), respectively, in the rituximab group and 6.05 ng/mL (SD, 10.08 ng/mL) and 5.1 ng/mL (SD, 9.06 ng/mL), respectively, in the placebo group. The decrease was not larger with rituximab than placebo (−2.20 ng/mL [SD, 6.07 ng/mL] vs. −0.89 ng/mL [SD, 6.82 ng/mL]; $P = 0.38$).

Adverse Events

Table 3 reports adverse events, severe adverse events, and discontinuation due to adverse events. Infusion reactions were significantly more common in the rituximab group (**Appendix Table 3**, available at www.annals.org). The only other significant between-group difference occurred for the proportion of patients who had at least 1 respiratory disorder ($P = 0.014$) within 24 hours after an infusion. Shortness of breath ($n = 1$), dry cough ($n = 1$), sneezing ($n = 1$), or throat irritation ($n = 5$) were recorded in 7 patients receiving rituximab. Only 1 respiratory disorder was considered severe, and all patients improved after the infusion was decreased or stopped. One patient in the placebo group had an asthma attack within 15 days after the infusion. Two patients in the rituximab group and none in the placebo group developed purpura that was considered to be treatment-related within 15 days after an infusion. Rates of infection and severe infection were similar between groups (bronchitis and urinary and cutaneous infections were the more frequent manifestations in both groups), and no patients had opportunistic infections. In 2 patients receiving rituximab, cancer was diagnosed during routine investigations at 7 days (squamous cell carcinoma of the skin) and 38 days (breast cancer in a woman who died 1 year after inclusion) after enrollment. In 1 patient receiving placebo, superficial basal cell carcinoma was diagnosed 125 days after inclusion.

Table 1. **Baseline Characteristics of 120 Treated Patients**

Characteristic	Rituximab ($n = 63$)	Placebo ($n = 57$)
Mean (SD) age, y	52.9 (13.3)	55.6 (13.6)
Mean (SD) time since diagnosis, y	4.6 (4.8)	5.5 (6.5)
Mean (SD) time since first symptom, y	7.4 (5.8)	8.4 (7.6)
Women, *n* (%)	57 (90.5)	55 (96.5)
Mean (SD) VAS score, mm		
Global disease	68.9 (18.6)	69.4 (17.0)
Pain	49.7 (29.6)	57.5 (26.3)
Fatigue	70.9 (18.2)	66.9 (19.2)
Dryness	68.5 (23.1)	72.4 (18.1)
Mean (SD) dryness VAS score, mm		
Mouth	64.7 (24.8)	70.9 (23.4)
Eyes	61.4 (27.6)	60.5 (29.4)
Skin	48.8 (26.5)	54.7 (26.0)
Trachea	33.8 (29.0)	46.1 (31.9)
Ocular dryness, *n* (%)	60 (95.2)	51 (89.5)
Oral dryness, *n* (%)	63 (100)	55 (96.5)
Abnormal Schirmer test result, *n* (%)	24/39 (67.2)	28/36 (77.8)
Mean (SD) Schirmer test result, mm	11.3 (9.1)	11.5 (12.1)
Mean (SD) break-up time, s	5.4 (3.4)	5.9 (5.0)
Decreased salivary flow rate, *n* (%)	35/39 (89.7)	34/37 (91.9)
Mean (SD) salivary flow rate, mL/min	0.2 (0.4)	0.1 (0.1)
Focus score >1, *n* (%)	56 (88.9)	49 (86.0)
Anti-Ro/SSA or anti-La/SSB, *n* (%)	51 (81.0)	46 (80.7)
Hypergammaglobulinemia, *n* (%)	34 (54.0)	29 (50.9)
Mean (SD) immunoglobulin level, g/L	15.2 (5.1)	16.4 (7.8)
IgA	3.1 (1.9)	2.7 (1.3)
IgG	16.0 (5.7)	16.8 (8.0)
IgM	1.4 (0.7)	1.6 (2.6)
Mean (SD) SF-36 score		
PCS	37.5 (8.9)	34.9 (9.3)
MCS	36.5 (9.7)	36.5 (9.5)
Systemic signs, *n* (%)*		
Parotid gland enlargement	22 (34.9)	18 (31.6)
Pulmonary	7 (11.1)	9 (15.8)
Neurologic	9 (14.3)	8 (14.0)
Articular	19 (30.2)	14 (24.6)
Mean (SD) ESSDAI score	10.0 (6.9)	10.2 (6.8)
Mean (SD) physician-assessed global VAS score	56.7 (18.5)	55.4 (18.6)
Mean (SD) ESR, mm/h	27.9 (20.5)	30.4 (28.5)
Mean (SD) serum CRP level, mg/L	9.7 (20.0)	7.1 (9.6)
Steroid therapy, *n* (%)	17/53 (32.1)	17/47 (36.2)
Methotrexate, *n* (%)	10/53 (18.9)	7/47 (14.9)

CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ESSDAI = European League Against Rheumatism Sjögren Syndrome Disease Activity Index; MCS = Mental Component Summary subscore; PCS = Physical Component Summary subscore; SF-36 = Short Form-36 Health Survey; VAS = visual analogue scale.

* Included when prevalence was >5% of the total population as assessed by clinicians.

Table 2. **Comparison of Improvements in the Rituximab and Placebo Groups at Weeks 6, 16, and 24***

Variable	Week 6			
	Rituximab	Placebo	Difference (95% CI)	*P* Value
Patients with ≥30-mm improvement in VAS score, %†				
≥2 of 4 VAS‡	22.4	9.1	13.3 (0.8 to 25.8)	0.036
Global	15.8	8.0	7.8 (−8.6 to 24.1)	0.35
Pain	18.0	14.0	3.9 (−9.9 to 17.8)	0.57
Fatigue	34.7	8.2	26.6 (15.7 to 37.5)	<0.001
Dryness	16.6	8.6	8.0 (−3.7 to 19.7)	0.179
Mean improvement in ESSDAI score	0.8	1.0	−0.3 (−1.2 to 0.7)	0.60
Patients with physician-assessed improvements, %				
Disease activity	44.9	25.8	19.1 (4.4 to 33.7)	0.011
Systemic signs	7.8	18.0	−10.1 (−21.8 to 1.5)	0.089
Treatment efficacy	56.6	35.6	21.0 (9.3 to 32.7)	0.001
Mean improvements§				
Physician VAS, mm†	16.8	8.5	8.4 (4.2 to 12.5)	<0.001
Salivary flow rate, mL/min	0.01	0.02	−0.01 (−0.11 to 0.08)	0.80
Schirmer test result, mm	−0.4	−2.9	2.5 (0.0 to 5.0)	0.054
ESR, mm/h	2.4	2.8	−0.4 (−4.8 to 4.0)	0.84
Serum CRP level, mg/L	0.6	0.4	0.2 (−6.0 to 6.4)	0.95
IgG, mg/L	1.1	1.8	−0.7 (−2.3 to 0.9)	0.37
IgA, mg/L	0.3	−0.2	0.5 (0.1 to 1.0)	0.026
IgM, mg/L	0.2	0.0	0.2 (0.1 to 0.2)	0.004
C4 complement level, g/L × 10^{-2}	0.0	−0.1	0.1 (−0.1 to 0.3)	0.32
β_2-Microglobulin level, g/L × 10^{-4}	0.2	−0.2	0.4 (−0.4 to 1.1)	0.35
SF-36 score				
PCS	3.5	2.2	1.3 (−1.6 to 4.3)	0.36
MCS	5.1	2.8	2.2 (−2.5 to 6.9)	0.35

CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; ESSDAI = European League Against Rheumatism Sjögren Syndrome Disease Activity Index; MCS = Mental Component Summary subscore; PCS = Physical Component Summary subscore; SF-36 = Short Form-36 Health Survey; VAS = visual analogue scale.
* Statistically significant results are in bold.
† Scores range from 0 (none) to 100 mm (worst).
‡ Primary outcome at week 24.
§ Improvement is defined as a decrease from baseline to weeks 6, 16, and 24 for all variables except salivary flow rate, Schirmer test result, C4 complement level, β_2-microglobulin level, and SF-36 score.

DISCUSSION

In this randomized, double-blind, placebo-controlled trial, two 1-gram doses of rituximab given 2 weeks apart did not significantly increase the proportion of patients achieving the primary end point (≥30-mm decrease in ≥2 of 4 VAS scores at week 24). However, rituximab was associated with clinically significant improvements at week 6, suggesting transient efficacy that was not maintained throughout the 24-week period with our regimen. Fatigue was alleviated early, whereas effects on dryness were delayed. Thus, although our data provide some support for the efficacy of rituximab reported in 2 previous preliminary studies (10, 11), the size and duration of the benefit argue against treating pSS with rituximab.

Fatigue, which is a major source of disability in patients with pSS (24), was the symptom that responded best to rituximab therapy in our study. This cannot be ascribed to methylprednisolone treatment, which was identical in the 2 study groups. The VAS dryness score improved significantly among patients in the rituximab group, but by less than 30 mm. In the previous randomized, placebo-controlled trial, the stimulated total salivary flow rate was the primary outcome measure and was significantly improved by rituximab therapy (11); however, a baseline rate of at least 0.15 mL/min was an inclusion criterion. Whereas that trial (11) showed improvements in stimulated and unstimulated total salivary flow rates after 24 weeks of rituximab versus baseline, mean salivary flow rate was not improved in our rituximab group. Similarly, we found no effects of rituximab on other variables associated with dryness, such as the Schirmer test score for tear production or the Chisholm grade for salivary gland inflammation.

As assessed by using the ESSDAI, rituximab therapy had no significant effect on systemic pSS in our study. Similarly, the systemic signs assessed by the investigators did not improve in the rituximab group. The low baseline ESSDAI value may partially explain this result. Conversely, in a prospective cohort study of 28 patients with pSS (18), the ESSDAI score improved significantly with rituximab therapy and showed good external and internal validity.

The only adverse events seen more often with rituximab than placebo were infusion reactions within 24 hours

Table 2.— Continued

Week 16				Week 24			
Rituximab	Placebo	Difference (95% CI)	*P* Value	Rituximab	Placebo	Difference (95% CI)	*P* Value
26.3	17.0	9.3 (−1.5 to 20.0)	0.091	23.0	22.0	1.0 (−16.7 to 18.7)	0.91
20.5	18.2	2.4 (−11.2 to 16.0)	0.73	16.9	24.0	−7.1 (−19.1 to 4.9)	0.25
15.2	15.9	−0.7 (−8.6 to 7.2)	0.86	12.6	22.0	−9.4 (−26.7 to 8.0)	0.29
27.2	8.9	18.3 (4.1 to 32.6)	0.012	20.1	10.8	9.3 (−2.0 to 20.6)	0.105
21.1	13.6	7.5 (−5.4 to 20.4)	0.25	25.6	13.2	12.4 (−3.0 to 27.8)	0.114
1.6	2.0	−0.3 (−1.7 to 1.0)	0.66	1.2	1.7	−0.5 (−2.3 to 1.3)	0.57
41.6	30.6	10.9 (−3.7 to 25.6)	0.142	44.6	43.3	1.4 (−15.3 to 18.0)	0.87
16.8	14.2	2.6 (−9.1 to 14.4)	0.66	18.4	22.7	−4.3 (−16.4 to 7.9)	0.48
53.6	52.8	0.8 (−8.6 to 10.2)	0.87	48.8	56.4	−7.6 (−20.0 to 4.8)	0.23
16.2	12.6	3.6 (−1.9 to 9.2)	0.20	15.0	10.9	4.1 (−1.6 to 9.8)	0.157
−0.01	−0.03	0.02 (−0.07 to 0.11)	0.69	0.01	−0.04	0.04 (−0.04 to 0.12)	0.29
−0.6	−1.4	0.7 (−2.7 to 4.2)	0.67	0.0	−1.9	1.9 (−0.2 to 4.1)	0.080
3.6	−0.9	4.5 (−1.7 to 10.7)	0.155	6.4	2.7	3.7 (−1.8 to 9.1)	0.185
3.0	1.9	1.1 (−2.5 to 4.7)	0.55	1.9	2.2	−0.3 (−2.3 to 1.6)	0.74
1.6	0.7	0.9 (0.1 to 1.8)	0.021	1.7	0.5	1.2 (0.4 to 2.0)	0.003
0.4	−0.1	0.4 (0.0 to 0.9)	0.063	0.4	−0.2	0.5 (0.0 to 1.1)	0.047
0.3	0.0	0.2 (0.1 to 0.3)	<0.001	0.3	0.0	0.3 (0.2 to 0.4)	<0.001
0.2	−0.1	0.3 (0.0 to 0.5)	0.048	0.2	0.1	0.1 (−0.2 to 0.4)	0.55
1.0	−0.5	1.5 (0.6 to 2.4)	0.001	1.0	−0.6	1.6 (0.5 to 2.8)	0.004
3.2	2.2	1.1 (−1.8 to 3.9)	0.46	3.8	3.2	0.6 (−1.5 to 2.6)	0.58
3.2	0.8	2.3 (−0.6 to 5.2)	0.116	1.7	1.2	0.5 (−2.9 to 4.0)	0.76

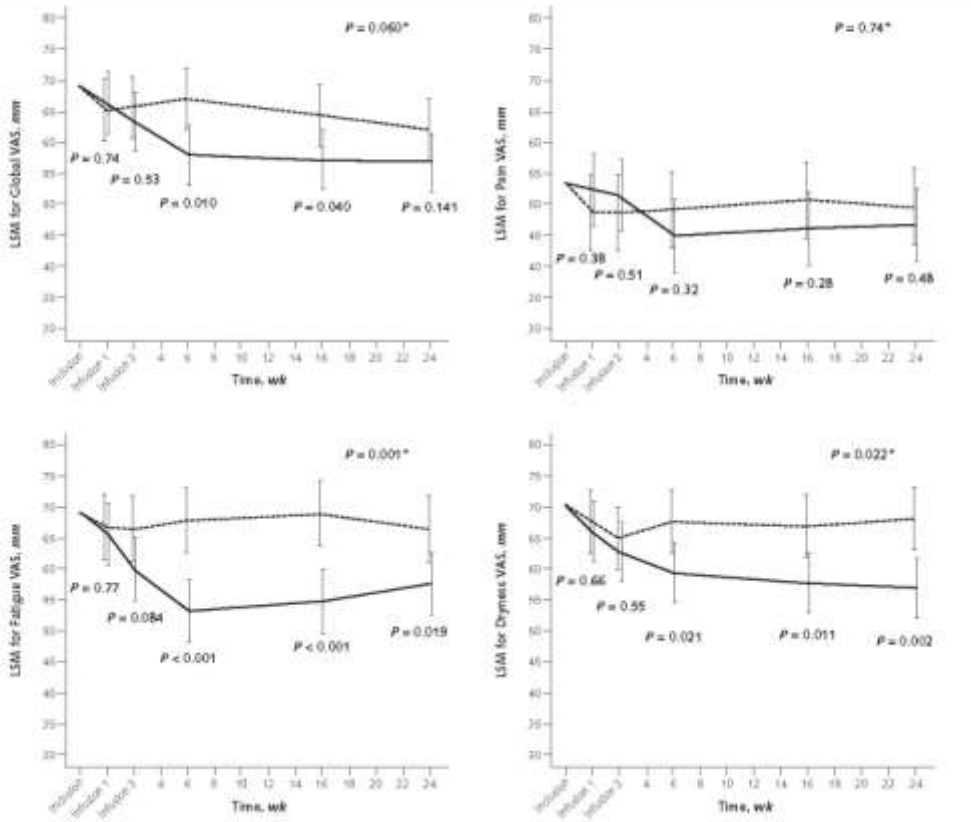
after an infusion. The occurrence of purpura in 2 patients who received rituximab was probably treatment-related. The difference in cutaneous adverse events between the groups was not significant, but this study was not powered to detect such a difference.

The timing of B-cell reconstitution is modulated by BAFF levels, and rituximab-induced B-cell depletion may result in decreased BAFF production (21). Serum BAFF levels were lower at week 24 than at baseline, but the difference was not statistically significant. Comparing serum BAFF levels at each time point to B-cell subsets in salivary gland infiltrates would be of interest because the results might help identify patients who are likely to respond. Similarly, the interferon response might help to predict the rituximab response because patients who do and do not respond to rituximab differ in the B-cell signaling pathway, extent of salivary gland infiltrates, and levels of interferon gene expression (25, 26).

In earlier studies, rituximab did not produce major therapeutic benefits in systemic lupus erythematosus, a disease whose pathophysiology largely depends on B cells (27). This finding suggests several points about the pathophysiologic process. First, plasmablasts have a long lifespan and exhibit limited susceptibility to rituximab, which may decrease the efficacy of rituximab therapy. Second, B cells in target organs, such as salivary glands, may show limited susceptibility to CD20 depletion. Finally, an imbalance between B cells and regulatory B cells in pSS may limit the therapeutic effects (28).

Our study has several limitations. First, the best outcome measure for assessing treatment efficacy in pSS is debatable. Clinical trials of potentially disease-modifying treatments in pSS have used various outcome measures (9, 10, 29–31). The earliest trial evaluating biological therapy for pSS used an improvement of at least 20% in 2 of 3 criteria (VAS scores or objective measures for oral and ocular dryness) and improvement in serum IgG level or erythrocyte sedimentation rate (30). Two recent, small, double-blind, placebo-controlled trials found that rituximab improved VAS scores for fatigue and dryness (10, 11). The ESSDAI (17), although validated to assess the activity of systemic pSS, was not selected in our study as the primary end point because we included patients without systemic manifestations. Given the high cost and po-

Figure 2. LSMs and 95% CIs for VAS scores for global disease, pain, fatigue, and dryness after adjustment for baseline characteristics (mixed model) in the rituximab (*solid line*) and placebo (*dotted line*) groups.



LSM = least-squares mean; VAS = visual analogue scale.
* Global *P* values are based on type III test evaluated with longitudinal regression analyses.

tential adverse effects of rituximab therapy, we chose a large effect as our primary outcome measure—namely, an improvement of at least 30 mm in at least 2 of 4 VAS scores evaluating different disease domains. This primary outcome may have been insensitive to detect clinically important changes in symptoms. Second, the best interval for assessing treatment efficacy in pSS is unclear. All previous studies on the biology of pSS evaluated the primary outcome between weeks 10 and 24. None of these studies suggested differences in treatment effects over time. We chose 24 weeks for our primary end point, an interval that seems consistent with the kinetics of rituximab's effects established in patients with rheumatoid arthritis. Third, patients had a low baseline activity score (mean ESSDAI score, 10.1), and we cannot exclude a better effect of the treatment in more active pSS. The study drug was prepared at hospital pharmacies in a manner that ensured blinding of the nurses, physicians, and patients. A few patients had infusion reactions, with no difference between the rituximab and placebo groups except for respiratory disorders and purpura. Therefore, the proportion of patients who may have guessed which treatment they received would have been small. In contrast, although corticosteroids given before infusions may modify the disease course over 1 month, the effect is probably not significant over a longer period.

Table 3. **Deaths, Adverse Events, Serious Adverse Events, and Discontinuations due to Adverse Events During the 24-Wk Study Period**

Variable	Patients, *n* (%)	
	Rituximab (*n* = 63)	Placebo (*n* = 57)
Death	0 (0)	0 (0)
Any adverse event	55 (87.3)	53 (93.0)
Infection	33 (52.4)	30 (52.6)
Any serious adverse event	13 (20.6)	8 (14.0)
Infection	2 (3.2)	5 (8.8)
Type of adverse event after first infusion*	5 (7.9)	1 (1.8)
Purpura†	1 (1.6)	–
Cytopenia†	1 (1.6)	–
Allergy (cutaneous)†	1 (1.6)	–
Allergy (respiratory)†	1 (1.6)	–
Hyperglycemia†	1 (1.6)	–
Headache†	–	1 (1.8)

* These patients did not receive the second infusion.
† Adverse event.
‡ Serious adverse event.

In conclusion, our data do not support the use of rituximab therapy in many patients with recent-onset or systemic pSS. Nevertheless, rituximab induced several significant improvements in patients with recent-onset or systemic pSS. Fatigue was alleviated rapidly, whereas effects on dryness were delayed. The only adverse events seen more often with rituximab than placebo were infusion reactions within 24 hours after an infusion.

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Appendix Table 1. **Sensitivity Analyses: Percentage of Patients With ≥30-mm Improvement for Each VAS and for ≥2 of 4 VASs***

Analysis	Week 6				Week 16				Week 24			
	Rituximab, %	Placebo, %	Absolute Difference (95% CI), *percentage points*	*P* Value	Rituximab, %	Placebo, %	Absolute Difference (95% CI), *percentage points*	*P* Value	Rituximab, %	Placebo, %	Absolute Difference (95% CI), *percentage points*	*P* Value
≥2 of 4 VASs†												
Multiple imputation (primary analysis)	22.4	9.1	13.3 (0.8 to 25.8)	0.036	26.3	17.0	9.3 (−1.5 to 20.0)	0.091	23.0	22.0	1.0 (−16.7 to 18.7)	0.91
Missing data regarded as nonresponse to treatment	19.1	7.1	12.1 (0.2 to 23.9)	0.047	23.9	15.7	8.2 (−1.1 to 17.5)	0.082	21.5	20.5	1.0 (−15.1 to 17.2)	0.90
Available data	20.4	7.3	13.1 (0.0 to 25.8)	0.045	25.6	16.4	9.3 (0.0 to 19.1)	0.064	21.9	20.9	1.0 (−16.1 to 18.2)	0.91
Global VAS												
Multiple imputation (primary analysis)	15.8	8.0	7.8 (−8.6 to 24.1)	0.35	20.5	18.2	2.4 (−11.2 to 16.0)	0.73	16.9	24.0	−7.1 (−19.1 to 4.9)	0.25
Missing data regarded as nonresponse to treatment	13.3	6.2	7.1 (−8.9 to 23.0)	0.39	18.5	16.6	1.9 (−11.2 to 15.1)	0.78	14.6	21.3	−6.7 (−17.0 to 3.5)	0.199
Available data	13.9	6.0	7.9 (−9.3 to 25.1)	0.37	20.4	17.4	2.9 (−10.6 to 16.5)	0.67	15.6	22.8	−7.2 (−18.2 to 3.7)	0.195
Pain VAS												
Multiple imputation (primary analysis)	18.0	14.0	3.9 (−9.9 to 17.8)	0.57	15.2	15.9	−0.7 (−8.6 to 7.2)	0.86	12.6	22.0	−9.4 (−26.7 to 8.0)	0.29
Missing data regarded as nonresponse to treatment	15.9	12.4	3.6 (−9.7 to 16.8)	0.60	14.3	15.7	−1.5 (−9.5 to 6.5)	0.71	11.6	21.5	−9.8 (−26.6 to 6.9)	0.25
Available data	16.8	12.8	3.9 (−9.8 to 17.7)	0.58	15.2	16.3	−1.1 (−9.0 to 6.8)	0.79	11.4	21.4	−10.0 (−27.2 to 7.3)	0.26
Fatigue VAS												
Multiple imputation (primary analysis)	34.7	8.2	26.6 (15.7 to 37.5)	<0.001	27.2	8.9	18.3 (4.1 to 32.6)	0.012	20.1	10.8	9.3 (−2.0 to 20.5)	0.105
Missing data regarded as nonresponse to treatment	33.0	6.9	26.1 (15.5 to 36.6)	<0.001	26.5	8.6	17.9 (5.2 to 30.5)	0.006	17.3	8.7	8.6 (−2.2 to 19.5)	0.119
Available data	34.4	7.0	27.4 (17.0 to 37.8)	<0.001	27.8	8.7	19.2 (5.7 to 32.6)	0.005	18.2	9.2	9.0 (−2.1 to 20.2)	0.113
Dryness VAS												
Multiple imputation (primary analysis)	16.6	8.6	8.0 (−2.7 to 19.7)	0.179	21.1	13.6	7.5 (−5.4 to 20.4)	0.25	25.6	13.2	12.4 (−2.0 to 27.8)	0.114
Missing data regarded as nonresponse to treatment	13.3	7.9	5.5 (−2.7 to 14.6)	0.24	21.3	13.3	8.0 (−3.8 to 19.8)	0.183	24.1	10.8	13.3 (0.0 to 26.5)	0.051
Available data	13.5	7.8	5.7 (−2.9 to 15.3)	0.24	21.5	13.0	8.5 (−2.9 to 20.9)	0.180	25.2	11.5	13.7 (−1.2 to 28.5)	0.071

VAS = visual analogue scale.
* Statistically significant results are in bold.
† Primary outcome at week 24.

Appendix Table 2. **Disease Activity in Each ESSDAI Domain in the Rituximab and Placebo Groups at Weeks 6, 16, and 24***

Domain	Baseline		Week 6		Week 16		Week 24	
	Rituximab (*n* = 63)	Placebo (*n* = 57)	Rituximab (*n* = 61)	Placebo (*n* = 56)	Rituximab (*n* = 60)	Placebo (*n* = 55)	Rituximab (*n* = 61)	Placebo (*n* = 54)
Constitutional	None: 47 Low: 5 *Moderate:* 11	None: 41 Low: 4 *Moderate:* 12	None: 51 Low: 0 *Moderate:* 10	None: 45 Low: 0 *Moderate:* 11	None: 48 Low: 1 *Moderate:* 11	None: 41 Low: 0 *Moderate:* 14	None: 48 Low: 2 *Moderate:* 11	None: 41 Low: 0 *Moderate:* 13
Lymphadenopathy	None: 59 Low: 3 *Moderate:* 1	None 54 Low: 3 *Moderate:* 0	None: 57 Low: 4 *Moderate:* 0	None: 54 Low: 2 *Moderate:* 0	None: 58 Low: 2 *Moderate:* 0	None: 54 Low: 0 *Moderate:* 1	None: 58 Low: 2 *Moderate:* 1	None: 52 Low: 2 *Moderate:* 0
Glandular	None: 46 Low: 10 *Moderate:* 8	None: 42 Low: 6 *Moderate:* 9	None: 44 Low: 13 *Moderate:* 4	None: 40 Low: 12 *Moderate:* 4	None: 47 Low: 11 *Moderate:* 2	None: 42 Low: 9 *Moderate:* 4	None: 47 Low: 9 *Moderate:* 5	None: 40 Low: 8 *Moderate:* 6
Articular	None: 32 Low: 12 *Moderate:* 13 *High:* 5	None: 30 Low: 14 *Moderate:* 9 *High:* 4	None: 33 Low: 12 *Moderate:* 13 *High:* 3	None: 31 Low: 16 *Moderate:* 7 *High:* 2	None: 33 Low: 17 *Moderate:* 8 *High:* 2	None: 31 Low: 16 *Moderate:* 6 *High:* 2	None: 36 Low: 16 *Moderate:* 5 *High:* 4	None: 32 Low: 14 *Moderate:* 4 *High:* 4
Cutaneous	None: 58 Low: 1 *Moderate:* 2 *High:* 2	None: 55 Low: 0 *Moderate:* 1 *High:* 1	None: 59 Low: 0 *Moderate:* 1 *High:* 1	None: 54 Low: 0 *Moderate:* 1 *High:* 1	None: 59 Low: 0 *Moderate:* 1 *High:* 0	None: 53 Low: 0 *Moderate:* 0 *High:* 2	None: 59 Low: 0 *Moderate:* 2 *High:* 0	None: 53 Low: 0 *Moderate:* 0 *High:* 1
Pulmonary	None: 52 Low: 10 *Moderate:* 1	None: 40 Low: 11 *Moderate:* 6	None: 49 Low: 11 *Moderate:* 1	None: 40 Low: 12 *Moderate:* 4	None: 49 Low: 11 *Moderate:* 0	None: 44 Low: 9 *Moderate:* 2	None: 49 Low: 11 *Moderate:* 1	None: 43 Low: 8 *Moderate:* 3
Renal	None: 57 Low: 1 *Moderate:* 0 *High:* 5	None: 56 Low: 0 *Moderate:* 0 *High:* 1	None: 56 Low: 1 *Moderate:* 0 *High:* 5	None: 55 Low: 0 *Moderate:* 0 *High:* 1	None: 55 Low: 1 *Moderate:* 0 *High:* 4	None: 55 Low: 0 *Moderate:* 0 *High:* 0	None: 55 Low: 1 *Moderate:* 0 *High:* 5	None: 53 Low: 0 *Moderate:* 0 *High:* 1
Muscular	None: 61 Low: 1 *Moderate:* 1	None: 56 Low: 1 *Moderate:* 0	None: 59 Low: 1 *Moderate:* 1	None: 55 Low: 1 *Moderate:* 0	None: 58 Low: 1 *Moderate:* 1	None: 54 Low: 1 *Moderate:* 0	None: 59 Low: 1 *Moderate:* 1	None: 53 Low: 1 *Moderate:* 0
PNS	None: 54 Low: 4 *Moderate:* 4 *High:* 1	None: 47 Low: 2 *Moderate:* 8 *High:* 0	None: 52 Low: 3 *Moderate:* 6 *High:* 0	None: 46 Low: 2 *Moderate:* 8 *High:* 0	None: 51 Low: 4 *Moderate:* 5 *High:* 0	None: 46 Low: 2 *Moderate:* 7 *High:* 0	None: 51 Low: 7 *Moderate:* 3 *High:* 0	None: 46 Low: 3 *Moderate:* 5 *High:* 0
CNS	None: 63 Low: 0 *Moderate:* 0	None: 57 Low: 0 *Moderate:* 0	None: 61 Low: 0 *Moderate:* 0	None: 56 Low: 0 *Moderate:* 0	None: 60 Low: 0 *Moderate:* 0	None: 55 Low: 0 *Moderate:* 0	None: 61 Low: 0 *Moderate:* 0	None: 54 Low: 0 *Moderate:* 0
Hematologic	None: 39 Low: 22 *Moderate:* 2	None: 34 Low: 18 *Moderate:* 5	None: 34 Low: 22 *Moderate:* 5	None: 35 Low: 18 *Moderate:* 3	None: 33 Low: 22 *Moderate:* 5	None: 35 Low: 16 *Moderate:* 4	None: 36 Low: 22 *Moderate:* 3	None: 34 Low: 17 *Moderate:* 3
Biological	None: 27 Low: 19 *Moderate:* 17	None: 24 Low: 15 *Moderate:* 18	None: 32 Low: 12 *Moderate:* 17	None: 25 Low: 13 *Moderate:* 18	None: 30 Low: 9 *Moderate:* 21	None: 22 Low: 16 *Moderate:* 17	None: 29 Low: 12 *Moderate:* 20	None: 20 Low: 17 *Moderate:* 17

CNS = central nervous system; ESSDAI = European League Against Rheumatism Sjögren Syndrome Disease Activity Index; PNS = peripheral nervous system.

Appendix Table 3. **Adverse Events and Serious Adverse Events During the 24-Wk Study Period and Postinfusion Periods**

System Organ Class	Patients With Adverse Event (Serious Adverse Event), n					
	During Study Period		Within 15 d of Infusion		Within 24 h of Infusion	
	Rituximab (*n* = 63)	Placebo (*n* = 57)	Rituximab (*n* = 63)	Placebo (*n* = 57)	Rituximab (*n* = 63)	Placebo (*n* = 57)
General disorders and administration site conditions	11 (2)	12 (0)	7 (1)	12 (0)	6 (0)	10 (0)
Skin and subcutaneous tissue disorders	15 (3)	12 (0)	10 (3)	9 (0)	4 (1)	6 (0)
Nervous system disorders	14 (3)	9 (0)	11 (0)	7 (0)	4 (0)	5 (0)
Respiratory, thoracic, and mediastinal disorders	8 (1)	8 (1)	7* (1)	1 (0)	7† (1)	0 (0)
Gastrointestinal disorders	19 (0)	16 (0)	8 (0)	5 (0)	3 (0)	2 (0)
Infections and infestations	33 (2)	30 (5)	10 (0)	5 (0)	3 (0)	2 (0)
Vascular disorders	6 (1)	6 (0)	2 (0)	3 (0)	2 (0)	3 (0)
Musculoskeletal and connective tissue disorders	18 (1)	11 (0)	2 (0)	7 (0)	0 (0)	3 (0)
Blood and lymphatic system disorders	3 (2)	3 (0)	3 (1)	1 (0)	1 (0)	1 (0)
Ear and labyrinth disorders	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Endocrine disorders	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Eye disorders	3 (0)	2 (0)	1 (0)	1 (0)	1 (0)	0 (0)
Immune system disorders	2 (1)	1 (0)	2 (0)	0 (0)	1 (0)	0 (0)
Psychiatric disorders	3 (0)	1 (0)	1 (0)	1 (0)	0 (0)	1 (0)
Renal and urinary	0 (0)	3 (1)	0 (0)	1 (0)	0 (0)	0 (0)
Benign, malignant, or unspecified neoplasm	2 (2)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Surgical and medical procedures	5 (1)	3 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Reproductive and breast disorders	3 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oral fungal infection	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Investigations	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Injury, poisoning, or procedural complications	5 (0)	4 (0)	0 (0)	1 (0)	0 (0)	0 (0)
Metabolism and nutrition disorders	3 (1)	1 (1)	1 (0)	0 (0)	1 (0)	0 (0)
Total	98 (13)	53 (8)	41 (5)	37 (0)	27 (2)	26 (0)

* $P = 0.064$ (Fisher exact test).
† $P = 0.014$ (Fisher exact test).

ABSTRACT NUMBER: 11L

Preliminary Results of a Double-Blind Randomised Trial of Rituximab Anti-B-Cell Therapy in Patients with Primary Sjogrens Syndrome

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SESSION INFORMATION

Date: Tuesday, November 10, 2015

Session Title: ACR Late-breaking Abstract Poster Presentations

Session Type: ACR Late-breaking Abstract Session

Session Time: 9:00AM-11:00AM

Background/Purpose: Evidence from open-label and observational studies support anti-B-cell therapy in patients with primary Sjogren's Syndrome (PSS). The TRACTISS trial aimed to determine the extent to which rituximab improves symptoms of fatigue and oral dryness in patients with PSS.

Methods: Multicentre, randomised, parallel group, double-blind, placebo-controlled trial. Patients with PSS, and symptomatic fatigue and oral dryness were recruited from 25 rheumatology clinics in the UK from June 2012 to January 2014. At weeks 0, 2, 24 and 26, patients received pre- and post-infusion corticosteroids and either placebo (P) IV or rituximab (R) IV (1000mg in 250mL). Intervention was decided by 24hr central telephone minimisation service. Primary endpoint was the proportion of patients achieving 30% reduction in either fatigue or oral dryness at 48 weeks,

measured by Visual Analogue Scale (VAS). Other outcomes included VAS scales for fatigue or oral dryness separately, global assessment of PSS activity, pain, ocular and overall dryness, as well as salivary and lachrymal flow rates, quality of life, EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient Reported Index (ESSPRI). Patients and physicians were blinded to the patient's allocation. ISRCTN 65360827

Results:

All patients (n=133) randomised to P (n=66) and to R (n=67) were included in the primary analysis. 55 P and 54 R patients received all 4 infusions in full. Mean age was 54 years, 93% of patients were female, mean ESSDAI was 5.7 and mean time since diagnosis was 5.7 years. Among complete cases at 48 weeks, 21/56 P and 24/61 R patients achieved the primary endpoint. After multiple imputation of missing outcomes, response rates were 36.8% (P) and 39.8% (R) (adjusted odds ratio 1.13, 95% confidence interval 0.50-2.55). There were no significant differences in any outcome measure, except unstimulated salivary flow: P patients deteriorated compared to R patients with a significant relative difference seen after Week 24. There were more adverse events reported in total for R (275 P vs 325 R), but no difference in serious adverse events. (10 vs 10) One serious infusion reaction (R) and one serious anaphylaxis (P) occurred in one patient each.

Conclusion: TRACTISS is the largest randomised trial of biologic therapy in PSS. No improvement in symptoms was seen in the Rituximab arm (unlike the TEARS study) but modest benefit for Rituximab in salivary flow was observed.

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Table 1: Summary of results (* denotes values were log-transformed before analysis, and results back-transformed for presentation, ** denotes effects as a ratio, rather than a difference)

	24 Weeks			48 Weeks		
	Placebo	Rituximab	Difference	Placebo	Rituximab	Difference
Unadjusted Complete Case analyses (95% Confidence Intervals)						
Fatigue VAS Response rates (%)	30.5 (19.2, 43.9)	29.5 (18.5, 42.6)	-1.0 (-18.6, 17.0)	26.8 (15.8, 40.3)	29.5 (18.5, 42.6)	2.7 (-15.5, 20.7)
Oral Dryness VAS Response rates (%)	22.0 (12.3, 34.7)	21.3 (11.9, 33.7)	-0.7 (-18.6, 17.0)	17.9 (8.9, 30.4)	31.1 (19.9, 44.3)	13.3 (-4.9, 30.9)
Primary Endpoint (Either fatigue or oral dryness) response rates (%)	37.3 (25.0, 50.9)	34.4 (22.7, 47.7)	-2.9 (-20.2, 15.3)	37.5 (24.9, 51.5)	39.3 (27.1, 52.7)	1.8 (-16.3, 19.9)

Mixed Model Estimates – adjusted for baseline values (95% Confidence Intervals)						
Fatigue VAS (0-100mm, 100=Severe)	64.5 (58.2, 71.5)	69.5 (63.7, 75.4)	4.7 (-2.9, 12.2)	65.8 (59.3, 72.2)	67.9 (61.3, 74.4)	2.1 (-5.9, 10.1)
Oral Dryness VAS (0-100mm, 100=Severe)	70.1 (63.9, 76.4)	70.2 (63.7, 76.7)	0.1 (-7.5, 7.6)	70.5 (64.5, 76.4)	66.4 (59.2, 73.7)	-4.1 (-12.0, 3.9)
Unstimulated Salivary Flow (mL/15min)*	0.66 (0.51, 0.87)	0.83 (0.64, 1.08)	1.25** (0.91, 1.72)	0.59 (0.45, 0.77)	1.00 (0.76, 1.31)	1.71** (1.23, 2.37)
ESSPRI (0-10, 10=Severe)	5.8 (5.3, 6.2)	6.3 (5.8, 6.8)	0.6 (0.01, 1.09)	5.7 (5.2, 6.2)	6.3 (5.7, 6.9)	0.5 (-0.1, 1.2)
ESSDAI (0-123, 123=Maximal activity)*	4.4 (3.6, 5.4)	4.1 (3.3, 5.2)	0.9** (0.7, 1.2)	4.5 (3.5, 5.8)	3.4 (2.7, 4.4)	0.8** (0.6, 1.0)

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ABSTRACT

BACKGROUND: Primary Sjögren's Syndrome (pSS) is a systemic autoimmune disease that involves the exocrine glands and internal organs. pSS leads to destruction and loss of secretory function due to intense lymphoplasmacytic infiltration. Therapeutic options include mainly symptomatic and supportive measures, and traditional immunosuppressant drugs have shown no effectiveness in randomized trials. Rituximab (RTX) is a chimeric antibody anti-CD20 that leads to B cell depletion by diverse mechanisms. There is evidence that this drug may be effective for treating pSS. The objective of this systematic review was to evaluate Rituximabeffectiveness and safety for treating pSS.

METHODS AND FINDINGS: We conducted a systematic review of RCTs published until December 2015, with no language restriction. We registered a protocol on PlataformaBrasil (40654814.6.0000.5505) and developed search strategies for the following scientific databases: MEDLINE, EMBASE, CENTRAL and LILACS. We included adults with established pSS diagnosis and considered the use ofRTX as intervention and the use of other drugs or placebo as control. Four studies met our eligibility criteria: three with low risk of bias and one with uncertain risk of bias. The total number of participants was 276 (145 RTX, 131 placebo). We assessed the risk of bias of each included study and evaluated the following as primary outcomes: lacrimal gland function, salivary gland function, fatigue improvement and adverse events. We found no significant differences between the groups in the Schirmer test at week 24 meta-analysis (MD 3.59, 95% CI -2.89 to 10.07). Only one study evaluated the lissamine green test and reported a statistically significant difference between the groups at week 24 (MD -2.00, 95% CI -3.52 to -0.48). There was a significant difference between the groups regarding salivary flow rate (MD 0.09, 95% CI 0.02 to 0.16) and improvement in fatigue VAS at weeks 6 (RR 3.98, 95% CI 1.61 to 9.82) and week 16 (RR 3.08, 95% CI 1.21 to 7.80).

CONCLUSIONS: According to moderate quality evidence, the treatment with a single RTX course in patients with SSp presents discrete effect for improving lacrimal gland function. Low-quality evidence indicates the potential of this drug for improving salivary flow. According to low quality evidence, no differences were observed in the evaluation after 24 weeks regarding fatigue reduction (30% VAS), serious adverse events occurrence, quality of life improvement and disease activity. With a very low level of evidence, there was no improvement in oral dryness VAS evaluation.

APÊNDICE

Apêndice 1. Registro na Plataforma Brasil e aprovação no Comitê de ética e pesquisa local.

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Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efetividade do Rituximab na Síndrome de Sjögren primária
Pesquisador: Francine Bertolais do Valle Souza
Área Temática:
Versão: 1
CAAE: 40654814.6.0000.5505
Instituição Proponente: Universidade Federal de São Paulo - UNIFESP/EPM
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 940.142
Data da Relatoria: 27/01/2015

Apresentação do Projeto:
A síndrome de Sjögren primária é uma doença autoimune, sistêmica, que acomete as glândulas exócrinas e órgãos internos, apresenta intensa infiltração linfoplasmocitária principalmente do epitélio dos tecidos envolvidos, levando a destruição e perda da função secretora. É uma desordem multifatorial com participação genética, hormonal e de fatores externos que contribuem para o desenvolvimento da doença. Acomete principalmente mulheres na proporção de 9:1, com pico de incidência entre 40 e 60 anos mais pode ocorrer em qualquer idade.
Apresenta distribuição universal. As manifestações iniciais geralmente não são específicas e o diagnóstico demora de 6 a 10 anos para ser realizado a partir dos sintomas iniciais. Em torno de 50% dos pacientes podem tem envolvimento sistêmico pulmonar, renal, sistema nervoso central e periférico, hepático, pancreático e vasculares. Pacientes com SSp apresentam um largo espectro de alterações laboratoriais como citopenias, hipergamaglobulinemia, presença de anticorpos antinucleares mais especificamente anti-Ro e anti-La, fator reumatoide, crioglobulinas e hipocomplementemia. As opções terapêuticas incluem medidas sintomáticas e de suporte. 1.2 Descrição da intervenção O Rituximab (RTX) é um anticorpo quimérico anti-CD20 da superfície dos linfócitos B, existem evidências que esse medicamento possa ser efetivo no tratamento de manifestações específicas na SSp. 1.4 Justificativa Recentemente alguns estudos têm sugerido o

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uso da Pilocarpina, Cevilemine, Hidroxicloroquina e Rituximab como as principais abordagens terapêuticas na Síndrome de Sjögren. Os resultados dos estudos são controversos com relação à efetividade do medicamento principalmente em relação às diferentes manifestações clínicas.

Objetivo da Pesquisa:

O objetivo é realizar uma revisão sistemática com a metodologia da Colaboração Cochrane para avaliar a efetividade e segurança do Rituximab no tratamento da Síndrome de Sjögren primária.

Avaliação dos Riscos e Benefícios:

Não se aplica

Comentários e Considerações sobre a Pesquisa:

Trata-se de estudo com o objetivo acadêmico de Mestrado, vinculado ao Departamento/Disciplina Medicina/Medicina de Urgência Campus São Paulo, com orientação do prof. Virgínia Fernandes M Trevisani.

Local A presente proposta de pesquisa será conduzida dentro do Programa de pós-graduação em Medicina Interna e Terapêutica (Saúde Baseada em Evidências) da Universidade Federal de São Paulo (UNIFESP).
2.3 Amostra
Critérios de inclusão: Quanto ao tipo de estudo Ensaios clínicos randomizados. 2.3.1.2 Quanto aos participantes
Os participantes serão adultos a partir de 18 anos com o diagnóstico estabelecido de SSp pelo consenso Americano e Europeu de classificação de SSp de 2002.
2.3.1.3 Quanto à intervenção O Rituximab (RTX) é um anticorpo quimérico anti-CD20 da superfície dos linfócitos B, existem evidências que esse medicamento possa ser efetivo no tratamento demanifestações específicas na SSp.
2.3.1.4 Quanto aos desfechos (primários e secundários) Primários: -Melhora da
secreção lacrimal e salivar por questionários, VAS e avaliação objetiva do fluxo salivar e de testes oftalmológicos (Schirmer, corante verde de lissamina e fluoresceína). - Melhora da fadiga que será avaliada através do Fatigue Impact
Scale (PROFAD), Functional Assessment of Chronic Illness Therapy Fatigue Subscale (FACIT-Fatigue), demais questionários validados e pelo VAS de fadiga. - Efeitos adversos do medicamento (por contagem de eventos). Melhora das manifestações sistêmicas através de avaliação clínica, laboratorial e por exames funcionais e de imagem.

Secundários: - A atividade da doença através do EULAR Sjögren?s Syndrome Disease Activity Index (ESSDAI). - Apercepção dos sintomas da SSP pelo EULAR Sjögren?s Syndrome Patient Reported Index (ESSPRI). - Alterações nos
níveis dos linfócitos B, fator reumatoide, e Fator ativador Linfócito B (BAAF). - Qualidade de vida através do Medica Outcomes Study 36- Item Short Form Health Survey (SF36) ou demais questonários validados.
2.3.2 Fontes de informação - Busca eletrônica Localizaremos os artigos publicados nas bases de dados Cochrane Library, Medline via PubMed, LILACS e Embase. A localização das pesquisas em andamento será efetuada por meio das bases de registro
de ensaios clínicos ClinicalTrials.gov. - Outras fontes Será feito contato com os autores a respeito de informações não
detalhadas nos métodos dos artigos e efetuaremos busca manual em anais de congressos. 2.3.3 Seleção dos estudos
Os estudos identificados serão submetidos à leitura do título e resumo para avaliação de elegibilidade por dois avaliadores independentes. Os estudos serão classificados como elegíveis serão lidos em texto completo enquanto os
não-elegíveis serão descartados. Após leitura do texto completo, os estudos elegíveis serão excluídos, com suas razões especificadas, ou incluídos na revisão. A cada etapa da seleção dos estudos será realizada uma reunião de consenso entre os avaliadores para dirimir concordâncias. 2.4. Extração e coleta dos dados Os dados serão extraídos
por dois avaliadores que utilizarão um formulário padronizado para coleta com informações sobre participantes, intervenção, comparação e desfechos. 2.5. Método estatístico 2.5.1 Tipos de dados e medida dos efeitos Os dados contínuos serão analisados por meio de diferença de média ou diferença de média padronizada. Dados dicotômicos serão analisados por cálculo do risco relativo, odds ratio e/ou diferença de risco. Todas as análises serão efetuadas com cálculo de intervalo de confiança de 95%. 2.5.2 Unidades de análise A unidade de análise utilizada será o indivíduo. Nos casos onde estudos clusters foram identificados, os mesmos serão analisados à parte. Estudos do tipo cross over serão analisados apenas no trecho antes do cruzamento. Os efeitos adversos serão quantificados através da contagem de eventos. Análise de subgrupos Serão feitas análises de acordo com: - Comprometimento específico. -
Protocolo de administração da intervenção (cíclico, dose, comparação com outras drogas). - Tempo da doença. - Idade.
2.5.4 Análise de sensibilidade Os resultados serão submetidos à análise de sensibilidade de

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Continuação do Parecer: 940.142

acordo com: - Risco de viés dos estudos; - Heterogeneidade.

2.5.5 Avaliação do risco de viés O viés dos estudos incluídos será avaliado por meio do instrumento da Cochrane para avaliação do risco de viés estruturado em sete domínios, a saber: - Geração da sequência de randomização - Sigilo de alocação - Cegamento dos participantes - Cegamento dos avaliadores - Relato seletivo - Dados incompletos - Outros 2.5.6 Viés de publicação O viés de publicação será avaliado por meio do gráfico de funil uma vez atendida a condição do número mínimo de estudos necessários (n = 10).

Considerações sobre os Termos de apresentação obrigatória:

documentos obrigatórios apresentados (FOLHA DE ROSTO, PROJETO DE PESQUISA)

Recomendações:

nada consta

Conclusões ou Pendências e Lista de Inadequações:

Sem indequações

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

O CEP informa que a partir desta data de aprovação, é necessário o envio de relatórios semestrais (no caso de estudos pertencentes à área temática especial) e anuais (em todas as outras situações). É também obrigatória, a apresentação do relatório final, quando do término do estudo.

SAO PAULO, 28 de Janeiro de 2015

Assinado por:
Miguel Roberto Jorge
(Coordenador)

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Apêndice 2. Artigo elaborado após conclusão dessa tese e publicado na revista PLOS one (open access).

Disponível em: http://dx.plos.org/10.1371/journal.pone.0150749

PLOS | ONE

RESEARCH ARTICLE

Rituximab Effectiveness and Safety for Treating Primary Sjögren's Syndrome (pSS): Systematic Review and Meta-Analysis

Francine Bertolais do Valle Souza[1]*, Gustavo José Martiniano Porfirio[2], Brenda Nazaré Gomes Andriolo[3], Julia Vajda de Albuquerque[4], Virginia Fernandes Moça Trevisani[5,6]

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Abstract

Background

Primary Sjögren's Syndrome (pSS) is a systemic autoimmune disease that involves the exocrine glands and internal organs. pSS leads to destruction and loss of secretory function due to intense lymphoplasmacytic infiltration. Therapeutic options include mainly symptomatic and supportive measures, and traditional immunosuppressant drugs have shown no effectiveness in randomized trials. Rituximab (RTX) is a chimeric antibody anti-CD20 that leads to B cell depletion by diverse mechanisms. There is evidence that this drug may be effective for treating pSS. The objective of this systematic review was to evaluate Rituximab effectiveness and safety for treating pSS.

Methods and Findings

We conducted a systematic review of RCTs published until December 2015, with no language restriction. We registered a protocol on *Plataforma Brasil* (40654814.6.0000.5505) and developed search strategies for the following scientific databases: MEDLINE, EMBASE, CENTRAL and LILACS. We included adults with established pSS diagnosis and considered the use of Rituximab as intervention and the use of other drugs or placebo as control. Four studies met our eligibility criteria: three with low risk of bias and one with uncertain risk of bias. The total number of participants was 276 (145 RTX, 131 placebo). We assessed the risk of bias of each included study and evaluated the following as primary outcomes: lacrimal gland function, salivary gland function, fatigue improvement and adverse events. We found no significant differences between the groups in the Schirmer test at week 24 meta-analysis (MD 3.59, 95% CI -2.89 to 10.07). Only one study evaluated the lissamine green test and reported a statistically significant difference between the groups at week 24 (MD -2.00, 95% CI -3.52 to -0.48). There was a significant difference between the

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groups regarding salivary flow rate (MD 0.09, 95% CI 0.02 to 0.16) and improvement in fatigue VAS at weeks 6 (RR 3.98, 95% CI 1.61 to 9.82) and week 16 (RR 3.08, 95% CI 1.21 to 7.80).

Conclusions

According to moderate quality evidence, the treatment with a single RTX course in patients with SSp presents discrete effect for improving lacrimal gland function. Low-quality evidence indicates the potential of this drug for improving salivary flow. According to low quality evidence, no differences were observed in the evaluation after 24 weeks regarding fatigue reduction (30% VAS), serious adverse events occurrence, quality of life improvement and disease activity. With a very low level of evidence, there was no improvement in oral dryness VAS evaluation.

Introduction

Primary Sjögren's Syndrome (pSS) is a systemic autoimmune disease that involves the exocrine glands and internal organs. pSS leads to destruction and loss of secretory function due to intense lymphoplasmacytic infiltration [1,2]. Genetic, hormonal and external factors contribute to the development of this multifactorial disorder [1–3] which has a worldwide distribution and affects mainly women, in the ratio of 9:1. The incidence peak of pSS is between 40 and 60 years of age, although pSS can occur at any age [4,5].The first signs and symptoms of the pSS are usually nonspecific, therefore the diagnosis can take between 6 to 10 years to be established [6].

Around 50% of individuals with pSS may have systemic involvement including the pulmonary, renal, hepatic, pancreatic, vascular, central nervous and peripheral nervous systems [7]. Individuals with pSS present a large spectrum of alterations in laboratorial tests such as cytopenias, hypergammaglobulinemia, presence of anti-Ro/SSA and anti-La/SSB antinuclear antibodies, rheumatoid factor (RF), cryoglobulins and hypocomplementemia [8]. Therapeutic options include mainly symptomatic and supportive measures [9].

Rituximab (RTX) is a chimeric antibody anti-CD20 that leads to B cell depletion by diverse mechanisms. There is evidence that this drug may be effective for treating pSS [10]. However, the results of studies regarding RTX effectiveness are controversial, mainly due to different clinical manifestations [9–12].

The objective of this systematic review was to evaluate the effectiveness and safety of Rituximab for treating pSS.

Methods

We conducted a systematic review of the literature in the Department of Evidence-Based Health of the São Paulo Federal University (UNIFESP). We registered the protocol on the *Plataforma Brasil* register of the Brazilian Ministry of Health (S1 Protocol).

Search strategy

We developed search strategies including the following terms and synonyms: "rituximab", "CD20 antibody rituximab", "Mabthera", "Roche brand of rituximab", "rituxan", "Hoffmann-La Roche brand of rituximab", "IDEC brand of rituximab", "Genentech brand of rituximab",

"IDEC-C2B8 antibody", "IDEC-C2B8", "Sjogren's Syndrome", "Sjogren Syndrome", "Sjogrens Syndrome", "Syndrome Sjogren's", "Sicca Syndrome" and "Syndrome Sicca". We ran the search strategies until December 2015 with filters for RCT's in the following databases: Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE (via PubMed), EMBASE and LILACS.

Study selection

Two authors (FBVS and GJMP) independently screened titles and abstracts and evaluated the eligibility of the identified studies. They read the studies classified as possibly eligible in full text and decided which studies to include in the review. In case of language barrier, the authors submitted articles to a qualified translator, and in case of eligibility disagreements, a third author (VMFT) made the final decision. We discarded the non-eligible studies due to specified reasons.

We included only randomized controlled trials (RCTs) and excluded cluster or cross-over trials. We selected RCTs with participants over 18 years of age and with an established pSS diagnosis according to the 2002 American-European Revised Classification Criteria [13]. We considered the use of Rituximab as intervention and the use of other drugs or placebo as control.

We considered the following primary outcomes: lacrimal gland function, evaluated through the Schirmer test, lissamine green or fluorescin test and VAS; salivary gland function, evaluated through salivary flow rate and VAS; and fatigue evaluated through the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), VAS and the Profile of Fatigue and Discomfort (PROFAD). We also analysed the adverse events reported by authors.

We considered the following secondary outcomes: quality of life, measured through the Short Form-36 (SF-36) health survey or other validated instruments; disease activity, evaluated through the EULAR Sjögren's Syndrome Disease Activity Index (ESSDAI) [14]; alterations in laboratorial variables (B lymphocyte, immunoglobulin, RF and B lymphocyte activating factor —BAAF—levels); and symptom perception, evaluated through the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) [15].

Two authors (FBVS and GJMP) extracted data from the studies through a standardized form with information about the participants, intervention, comparison, outcomes and characteristics. We assessed the risk of bias in each included study as high, low or unclear through the Cochrane Collaboration's tool for assessing risk of bias [16], which is structured in seven domains: random sequence generation; allocation concealment; blinding of participants and personnel; blinding of outcome assessment; incomplete outcome data; selective reporting; and other sources of bias.

Data synthesis

We included the data assessed at week 24 from baseline on the Review Manager (Revman) 5.3.4 software. We analysed continuum data through mean difference and dichotomous data through risk ratio. We calculated all the effect measures with a 95% confidence interval. We assessed the statistical heterogeneity between studies through the I^2 statistic [17] and considered the presence of significant heterogeneity for values superior to 50%.

We planned subgroup analyses considering the organ specific commitment, intervention protocol (cycle, dosage, comparison to other drugs), time of disease and age. We also planned sensitivity analyses considering the risk of bias and heterogeneity between studies and the statistical model used in the meta-analyses. We evaluated the quality of evidence through the GRADE approach.

Results

We identified 126 records and included four studies [18–20] in the quantitative analysis (Fig 1).

We analysed each included study through the Cochrane Collaboration's tool for assessing risk of bias (Fig 2).

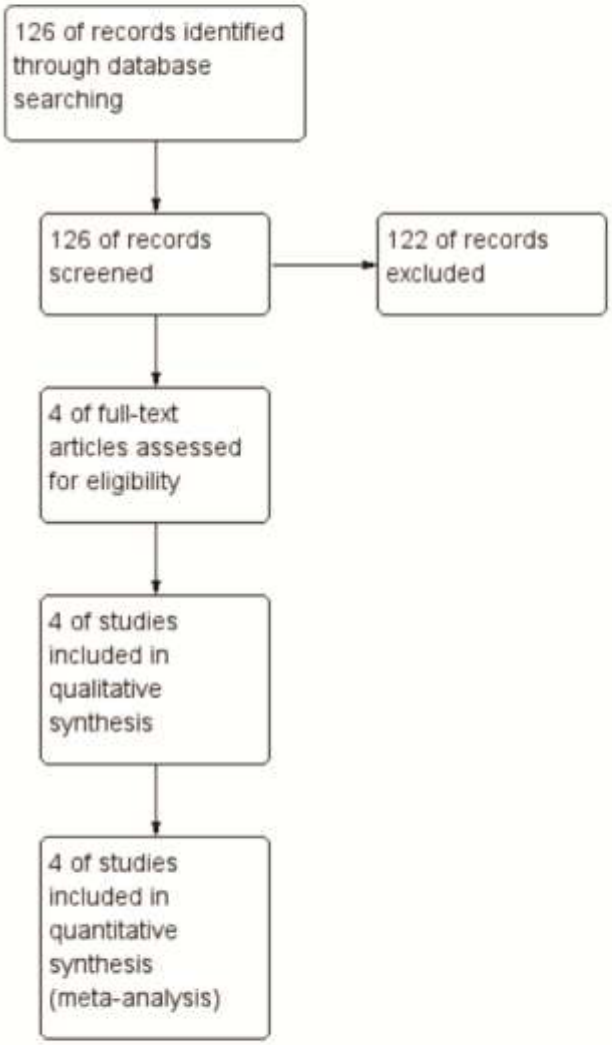

Fig 1. Flow chart of studies selection.

doi:10.1371/journal.pone.0150749.g001

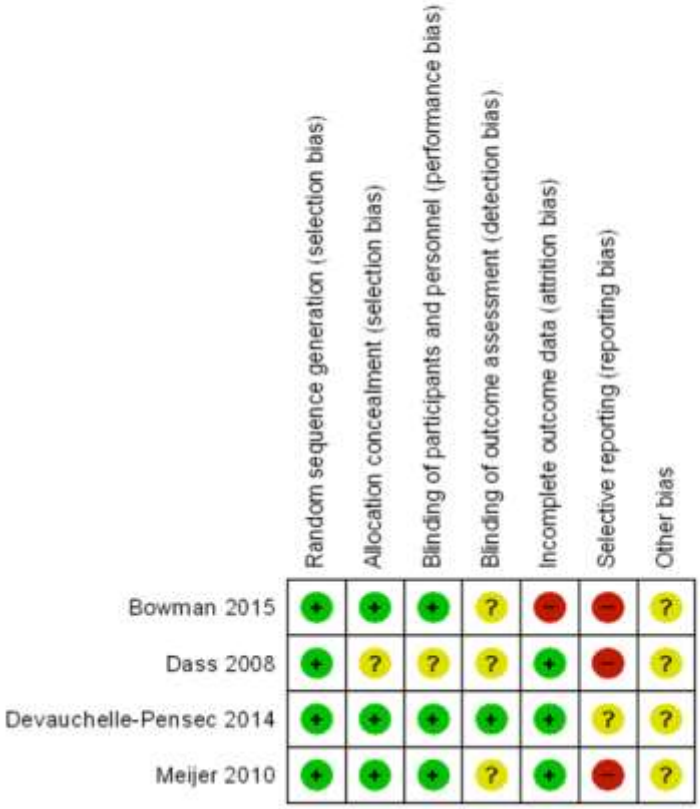

Fig 2. Risk of bias assessment of included studies.

doi:10.1371/journal.pone.0150749.g002

The included studies totalized 276 participants: 145 in the RTX group and 131 in the control group (placebo). All included studies [18–21] randomly assigned patients to the RTX group (two 1g RTX infusions: day 1 and day 15, single course) or control group (placebo infusion: single course). We analysed data assessed 24 weeks from baseline.

Primary Outcomes

Three of the included studies evaluated lacrimal gland function through the Schirmer test [18–20]. Dass et al. [18] reported no changes but presented no results. Thus, we included only two studies [19,20] in the Schirmer test at week 24 meta-analysis. Meijer et al. [19] reported lacrimal gland function improvement in the RTX group since baseline until week 48. Yet, they found no differences between the groups in the Schirmer test. Devauchelle-Pensec et al. [20] found no effects of RTX in variables associated to dryness such as the results for lacrimal production in the Schirmer test. We performed a fixed-effect model meta-analysis of this outcome

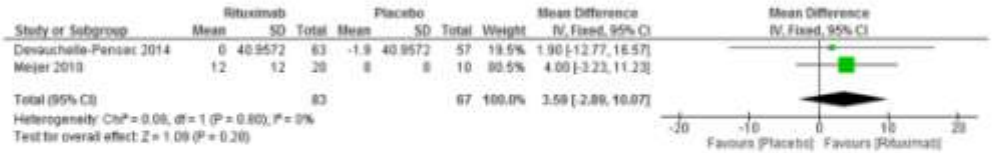

Fig 3. Rituximab X Placebo. Meta-analysis of the outcome Schirmer test at week 24.

doi:10.1371/journal.pone.0150749.g003

and observed no statistically significant differences between the RTX and placebo groups at week 24 (MD 3.59, 95% CI -2.89 to 10.07; Fig 3). We imputed the standard deviation data from Devauchelle-Pensec et al. [20] through *p* value and mean difference.

Meijer et al. [19] evaluated lacrimal gland function through the lissamine green test and found a significant difference between the RTX and placebo groups at week 24 (MD -2.00, 95% CI -3.52 to -0.48). Meijer et al. [19] also reported improvement of ocular dryness VAS scores in the RTX group since the baseline until week 48, whilst the scores in the placebo group only presented significant improvement after week 5. There was a significant difference in the average alteration of ocular dryness VAS between groups from baseline to week 24 (MD -27.00, 95% CI -46.28 to -7.72), week 36 (MD -24.0, 95% CI -44.5 to -3.5) and week 48 (MD -30.00, 95% CI -47.01 to -12.99).

All included studies reported analysis of salivary flow data [18–21]. Dass et al. [18] reported no changes but presented no quantitative data, thus we excluded this study from the salivary flow rate at week 24 meta-analysis. Meijer et al. [19] reported improvement of salivary flow rate in the RTX group at week 24 (MD 0.14, 95% CI 0.02 to 0.26), whilst Devauchelle-Pensec et al. [20] and Bowman et al. [21] observed no significant differences between the RTX and placebo group at week 24 (MD 0.05, 95% CI -0.04 to 0.14 and MD 0.17, 95% CI -0.07 to 0.41, respectively). We performed a fixed-effect model meta-analysis of the outcome salivary flow rate and demonstrated a statistically significant difference between the groups in favor of the RTX group at week 24 (MD 0.09, 95% CI 0.02 to 0.16; Fig 4).

Two [19–21] of the included studies evaluated oral dryness through VAS. Meijer et al. [19] reported improvement in the VAS score for all oral dryness symptoms in the RTX group. They found a statistically significant mean difference between the groups at week 24 (MD -30.00, 95% CI -50.50 to -9.50). We performed a random-effects model meta-analysis of this outcome and observed no statistically significant differences between the RTX and placebo groups at week 24 (MD -13.47, 95% CI -42.82 to 15.89; Fig 5). Bowman et al. [21] also evaluated oral dryness through VAS response rates (%) and found no significant differences between the groups (RR 0.93, 95% CI 0.45 to 1.93).

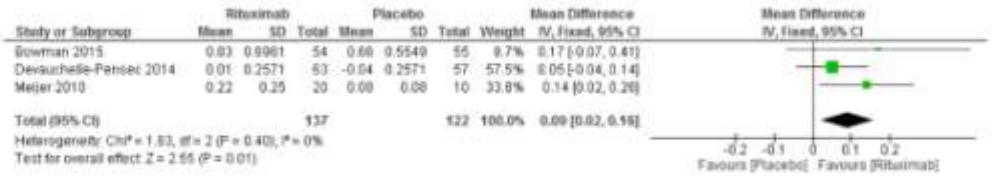

Fig 4. Rituximab X Placebo. Meta-analysis of the outcome salivary flow rate at week 24.

doi:10.1371/journal.pone.0150749.g004

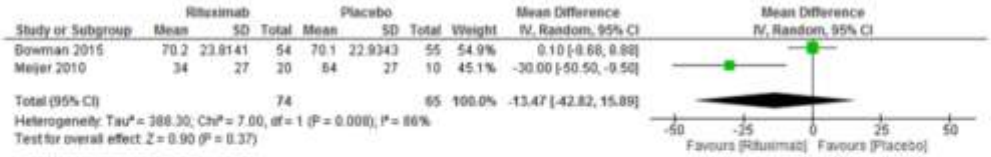

Fig 5. Rituximab X Placebo. Meta-analysis of the outcome oral dryness (VAS) at week 24.

doi:10.1371/journal.pone.0150749.g005

All included studies evaluated fatigue through VAS. Dass et al. [18], Devauchelle-Pensec et al. [20] and Bowman et al. [21] established up to 30% improvement as the primary endpoint. We found no significant differences between the groups in the meta-analysis of fatigue VAS 30% improvement at week 24 (RR 1.12, 95% CI 0.75 to 1.66; Fig 6). However, fatigue VAS results from Devauchelle-Pensec et al. [20] indicated a favorable response to RTX at week 6 (RR 3.98, 95% CI 1.61 to 9.82; Fig 6) and week 16 (RR 3.08, 95% CI 1.21 to 7.80; Fig 6). Bowman et al. [21], also reported fatigue outcome through VAS mean score (0-100mm, 100 = Severe) and found no significant differences between groups (MD 5.0, 95% CI -3.37 to 13.37). Only Dass et al. [18] evaluated fatigue through PROFAD and reported that there was a significant somatic fatigue domain improvement in the RTX group at week 24 ($p = 0.009$), but not in the placebo group ($p = 0.087$).

We evaluated the quality of evidence through the GRADE approach. The Schirmer test meta-analysis presented moderate quality. The salivary flow rate and fatigue VAS 30%

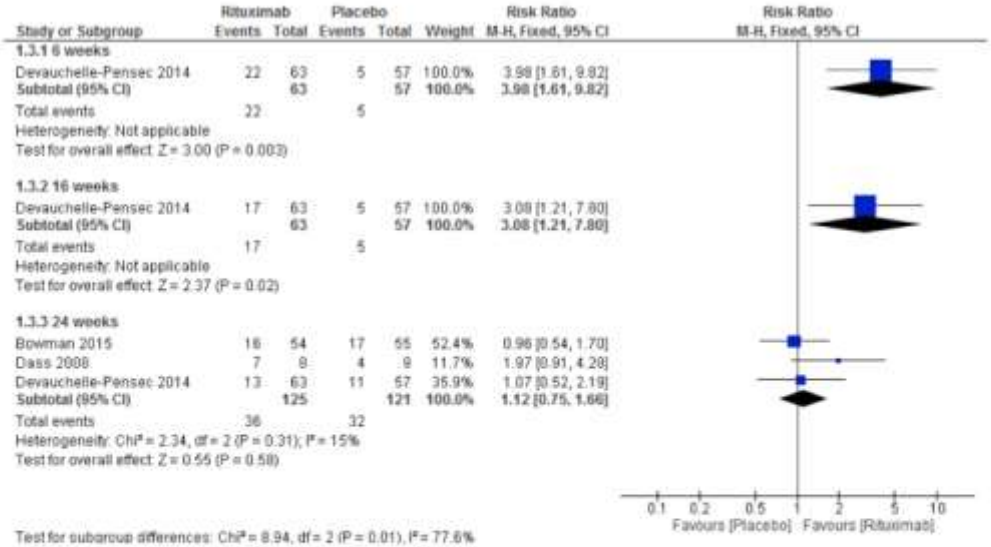

Fig 6. Rituximab X Placebo. Meta-analysis of the outcome fatigue VAS 30% improvement at week 24.

doi:10.1371/journal.pone.0150749.g006

Fig 7. Rituximab X Placebo. Meta-analysis of the outcome SF-36 mental component summary at week 24.

doi:10.1371/journal.pone.0150749.g007

improvement meta-analyses presented low quality evidence. The oral dryness VAS meta-analysis presented very low-quality evidence.

Secondary outcomes

Three of the included studies [18–20] evaluated quality of life through the SF-36 health survey but reported results differently. Meijer et al. [19] only reported the SF-36 total score and found no significant differences between the groups from baseline to week 48 (MD -5.00, 95% CI -17.15 to 7.15). Dass et al. [18] reported improvements in the mental health domain. Devauchelle-Pensec et al. [20] only described the mental and the physical component summary subscores and did not mention the SF-36 total score. They found no significant differences between the groups in the physical domain at week 24 (MD 0.60, 95% CI -16.90 to 18.10). Due to heterogeneity between studies, we performed a random-effects meta-analysis of mental health outcome at week 24 and found no significant differences between the groups (MD 10.85, 95% CI -15.26 to 36.97; Fig 7). This meta-analysis presented low-quality evidence according to the GRADE approach.

Devauchelle-Pensec et al. [20] and Bowman et al. [21] assessed disease activity through the ESSDAI. We performed a meta-analysis for this outcome and found no significant differences between the RTX and placebo groups (MD -0.30, 95% CI -1.40 to 0.79; Fig 8). According to the GRADE approach, this meta-analysis presented low quality evidence.

Dass et al. [18] demonstrated that in terms of laboratory results, there was a significant difference in RF reduction at week 24 favorable to the RTX group (MD 45, 95% CI 3.62 to 86.38). Devauchelle-Pensec et al. [20] mentioned they would analyse the RF as a secondary outcome, however, the result was not presented in the study.

Only Meijer et al. [19] described the analysis of B cells number. They observed an expressive decrease in the mean absolute number of B cells after the first RTX infusion and no significant alterations in the placebo group. They found statistically significant differences in the mean absolute number of B cells between the groups since baseline until weeks 5, 12, 24, 36 and 48 (MD -0.23, 95% CI -0.31 to -0.15).

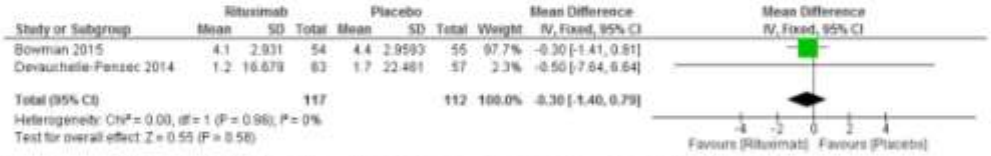

Fig 8. Rituximab X Placebo. Meta-analysis of the outcome disease activity assessed through the ESSDAI at week 24.

doi:10.1371/journal.pone.0150749.g008

Two studies evaluated immunoglobulin levels [18–20]. Dass et al. [18] reported a percentage reduction difference in favor of RTX (p = 0,05). Devauchelle-Pensec et al. [20] found a significant IgM reduction favorable to the RTX group (MD 0,30, 95% CI 0.13 to 0.47).

Only Bowman et al. [21] evaluated symptoms perception through the ESSPRI and found no significant differences between the RTX and placebo groups (MD 0.50, 95% CI -0.19 to 1.19).

Safety

All included studies reported adverse effects. Infusion-related reactions such as shiver, macular rash and purpura were more frequent in the RTX, as well as gastrointestinal, musculoskeletal and respiratory disorders. However, Meijer et al. [19] and Devauchelle-Pensec et al. [20] found similar infection rates between the RTX and placebo groups.

Meijer et al. [19] and Devauchelle-Pensec et al. [20] reported, respectively, that one and two RTX patients developed purpura within 15 days after infusion. Devauchelle-Pensec et al. [20] reported one occurrence in the placebo group.

Devauchelle-Pensec et al. [20] reported events such as shortness of breath, dry cough, sneezing or throat irritation in seven RTX patients, and found a significant difference between the groups in the proportion of patients with at least one respiratory disorder 24 hours after an infusion (p = 0.014). Only one of these events was considered serious and all patients improved after an infusion decrease or the treatment interruption. One patient in the placebo group had an asthma attack within 15 days after infusion [20].

Devauchelle-Pensec et al. [20] reported two cancer diagnoses in the RTX group during the investigations: one at day 7 from baseline (squamous cell carcinoma) and the other at day 38 (breast cancer; this patient died 1 year after inclusion in the study). One patient from the placebo group was diagnosed with superficial basal cell carcinoma 125 days after inclusion in the study [20].

Bowman et al. [21] reported occurrence of more adverse events in the RTX group than in placebo (275 Placebo vs 325 RTX). However, the adverse events considered serious were equal between groups (10 vs 10). The authors also reported just one serious infusion reaction (RTX) and one serious anaphylaxis (placebo).

Serious adverse events occurred in 25 participants of the RTX group [18,20,21] and in 18 participants of the placebo group [20,21]. We performed a fixed-effect meta-analysis for this outcome and found no significant differences between groups (RR 1.33, 95% CI 0.77 to 2.30; Fig 9). This meta-analysis presented low quality evidence according to the GRADE approach.

Discussion

The number of published articles about the chimeric antibody anti-CD20 (rituximab) in the treatment of pSS has been growing over time. However, most identified studies are case reports

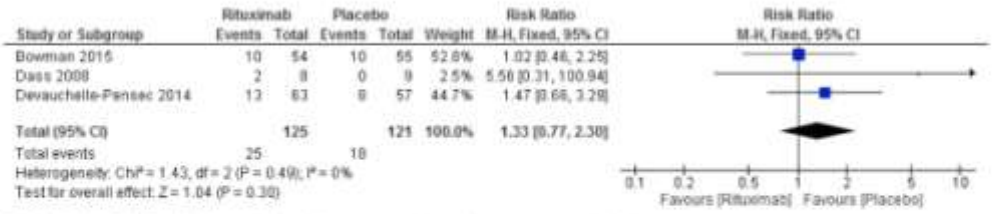

Fig 9. Rituximab X Placebo. Meta-analysis of the outcome serious adverse events at week 24.

doi:10.1371/journal.pone.0150749.g009

or series of specific systemic manifestations. Of the studies found, only four met the inclusion criteria of our study, and one [21] was published in the form of preliminary results.Carubbi et al. [22] recently published a trial comparing RTX to immunosupressants and demonstrated the superiority of RTX. However, due to methodological aspects, this study could not be included in this review.Until recently, the treatment of pSS included only symptomatic and supportive measures. RCTs with traditional immunosupressants have shown no efficacy in the treatment of this multi-systemic disorder [23–27].The frequently serious pSS systemic features, the probability of evolution to lymphoproliferative disease [28] and the progress in the recognition of the role of cytokines (such as IFN-γ and IL-2) and the abnormal B cell activation established a possibility of new therapeutic measures [29–31].

Patients with pSS present B cell hyperactivity, which suggests an important role of these cells in the pSS pathogenesis [30–31]. RTX acts directly against these cells. Thus, RTX is considered a potential treatment for pSS.

There is still no consensus regarding the best time interval to evaluate the pSS treatment efficacy [20]. We considered a treatment period of 24 weeks for the outcomes analyses since this interval was common to all included studies.

Systemic manifestations were not evaluated in the studies and the follow-up period was short, therefore it was not possible to analyze bad prognostic factors of the disease. Our main results were regarding ocular gland function, salivary gland function and fatigue. Two studies [20,21] evaluated the disease activity and found no significant differences between the groups. The action of RTX in the reduction of RF and B cell number was demonstrated by Dass et al. [18] and Meijer et al. [19], respectively.

Patients with pSS present high incidence of non-Hodgkin lymphoma, mainly mucosa lymphomas [32,33]. The use of RTX, associated or not to chemotherapy, is an option for the treatment of this complication in pSS [32]. However, since the included studies did not report the presence of patients with lymphoma, it was not possible to conclude about this aspect.

A significant increase in the risk of adverse effects, mainly infusion reactions and respiratory disorders, was noted for participants allocated to RTX compared to those given placebo in all included studies. However, infection rates were similar between the groups in three studies: Dass et al. [18], Devauchele-Pensec et al. [20] and Meijer et al. [19]. Bowman et al. [21] reported that the occurrence of adverse events considered serious was equal between groups.

This review highlights the difficulty and inadequacy of research in pSS since that there are only a few randomized studies about RTX that compare the effectiveness of this drug to placebo or other drugs. The included studies presented similar endpoints, however the evaluation of outcomes still needs standardization. It was not possible to perform a subgroup or sensitivity analysis considering factors other than the model of effect used in the meta-analyses.

We evaluated only one treatment cycle since the assessment at week 24 was performed in all studies. Although Bowman et al. [21] administrated two RTX courses, they found no significant differences in any outcomes measured, except for salivary flow rate, as we have found in this study.

The number of participants included in this study was limited, even in the meta-analyses, and there was no efficacy analysis for different systemic manifestations. Regarding the benefits observed, only the outcome salivary flow demonstrated evidence of improvement, and RTX demonstrated itself to be safe since there were no differences in the presence of serious adverse events compared to the placebo group. For clinical practice, it is necessary to ponder the benefits and harms of this intervention in the treatment of pSS.

Conclusion

According to moderate-quality evidence, the treatment with a single RTX course for patients with SSp presents discrete effect for improving lacrimal gland function. Low quality evidence indicates the potential of this drug for improving salivary flow. According to low quality evidence, no differences were observed in the evaluation after 24 weeks regarding fatigue reduction (30% VAS), serious adverse events occurrence, quality of life improvement and disease activity. With a very low level of evidence, there was no improvement of oral dryness VAS evaluation.

Supporting Information

S1 PRISMA checklist.
(DOC)

S1 Protocol. Registered on *Plataforma Brasil* (40654814.6.0000.5505).
(DOCX)

Author Contributions

Conceived and designed the experiments: FBVS VFMT. Performed the experiments: FBVS GJMP BNGA VFMT. Analyzed the data: FBVS GJMP VFMT. Contributed reagents/materials/analysis tools: FBVS GJMP JVA BNGA VFMT. Wrote the paper: FBVS JVA.

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Esta tese foi formatada de acordo com as seguintes orientações:

- Livro "Como escrever sua tese". Autoras: Edna Terezinha Rother e Maria Elisa Rangel Braga.
- DECS - descritores em ciências da saúde.
- Para abreviatura dos títulos de periódicos internacionais, consultamos: http://www.ncbi.nlm.nih.gov/